DIAGNOSTIC ACCURACY OF COLOUR DOPPLER ULTRASONOGRAPHY IN EVALUATION OF CERVICAL LYMPH NODES IN ORAL CANCER PATIENTS

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CERTIFICATE

Certified that the dissertation on "**Diagnostic accuracy of colour Doppler ultrasonography in evaluation of cervical lymph nodes in oral cancer patients**" done by **Dr. Suresh Kumar**, Post Graduate student (M.D.S.), **Branch IX Oral Medicine and Radiology**, Tamilnadu Government Dental College and Hospital, Chennai submitted to The Tamilnadu Dr. M.G.R. Medical University for partial fulfilment of the M.D.S. Degree Examination in April 2011, is a bonafide research work done under my guidance and supervision.

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ABBREVIATIONS

AJCC- AMERICAN JOINT COMMITTEE ON CANCER

CDVI-COLOUR DOPPLER VASCULARITY INDEX

CDUS-COLOUR DOPPLER ULTRASONOGRAPHY

FNAC-FINE NEEDLE ASPIRATION CYTOLOGY

PDUS-POWER DOPPLER ULTRASONOGRAPHY

PI-PULSATILITY INDEX

RI-RESISTIVITY INDEX

US-FNAC-ULTRASOUND GUIDED FINE NEEDLE ASPIRATION CYTOLOGY

USG-ULTRASONOGRAPHY

CONTENTS

1.	INTRODUCTION	1
2.	AIMS AND OBJECTIVES	23
3.	REVIEW OF LITERATURE	24
4.	MATERIALS & METHODS	40
5.	TABLES & CHARTS	46
6.	RESULTS & ANALYSIS	53
7.	DISCUSSION	62
8.	SUMMARY & CONCLUSION	73
9.	BIBLIOGRAPHY	76

INTRODUCTION

Cervical lymphadenopathy results from a vast group of disease whose broad categories can be easily recalled by the acronym ‘‘MIAMI’’, representing Malignancies, Infections, Autoimmune disorders, Miscellaneous and unusual conditions, and Iatrogenic causes.[17] Regional metastasis is an important factor in the treatment and prognosis of patients with oral cancer because the presence of metastatic node reduces the 5-year survival rate by 50% and the presence of another metastatic node on the contra-lateral side further reduces the survival rate by 25%.[3,4,38,70]

An understanding of the mechanism by which tumour cells establish metastasis is crucial for tumour biology.[29] Okura et al[55] suggested the possibility of suppression of cellular immune response in particular T-cell function in development of lymph node metastasis. Martinez–Gimeno et al[46] have mentioned micro-vascular invasion, grade of differentiation and tumour thickness as important risk factors for metastasis. Nakayama et al[52] stated that lymphatic vessel diameter and the mode of invasion may be the factors in the prediction of cervical lymph node metastases.

The management of oral cancer is based on the status of cervical lymph nodes so there is a need for an appropriate

investigative procedure which can differentiate between metastatic and reactive lymph nodes in patient with oral cancer to aid in the grading and staging of the oral cancer which determines the treatment plan, prognosis and morbidity.

The characteristic event in tumour formation is angiogenesis and therefore the morphologic and hemodynamic changes that occur in tumour vessels can be used as a clue to differentiate between metastatic and reactive nodes. Blood vessel morphology in metastatic nodes is usually deranged as the internal nodal architecture is destroyed by neoplastic infiltration and neo-vascularisation induced by angiogenesis factor, whereas inflammation causes dilatation of intranodal vessels due to local humoral agents.

All these intranodal vascular alterations provide the potential for diagnosis, if vascular changes can be reliably detected. Colour Doppler Ultrasound is one of the advancement in sonography which can helps in differentiating metastatic from reactive nodes based on these vascular changes.[53,5]

Colour Doppler ultrasound (CDUS) is a non-invasive procedure that can define the morphologic and vascular characteristics of the lymph node. The presence of intra-nodal vascularity, its distribution

and estimation of the intravascular resistance and spectral Doppler analysis are evaluated with colour Doppler.[46]

CDUS can be used to improve pre-operative staging of oral cancer. In selected cases this may direct the surgeons to convert the treatment plan to choose a more conservative neck dissection or to a more radical neck dissection.[29] Studies have shown that there is a general tendency of the clinician to over-diagnose the cervical lymph nodes as metastatic in patients with oral cancer. CDUS can also avoid this over-diagnosis and can prevent the unnecessary removal of nodes in each and every patient with oral cancer.[28,29]

Therefore this study was conducted to evaluate the possibility of using CDUS as an alternative investigative procedure in differentiating metastatic nodes from reactive nodes in patients with oral cancer. This study also showed the importance of CDUS in discovery of clinically non-palpable cervical lymph nodes and value of CDUS in minimizing the need for Fine needle aspiration cytology (FNAC).

NORMAL ANATOMY OF LYMPH NODE

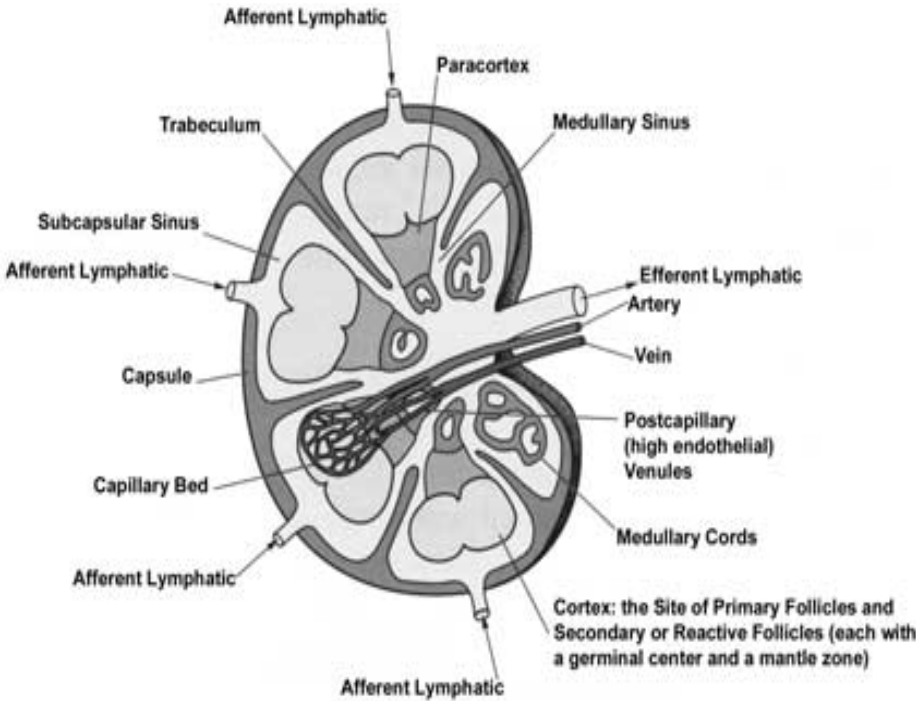
Cervical lymph nodes are located along the lymphatic channels of the neck. Each cervical lymph node has cortical and medullary regions, and is covered by a fibrous capsule.[21]The cortex consists of lymphocytes which are densely packed together to form spherical lymphoid follicles, whereas the medulla is composed of medullary trabeculae, medullary cords and medullary sinuses.[77]

The para-cortex is an intermediate area between the cortex and the medulla, where the lymphocytes return to the lymphatic system from the blood circulation. In the medulla of the lymph node, the medullary trabeculae, composed of dense connective tissue similar to the capsule, act as a framework extending from the capsule and guides blood vessels and nerves to different regions of the lymph node. The medullary cords and medullary sinuses are composed of reticulum cells. The medullary cords contain mainly plasma cells and small lymphocytes, whilst the medullary sinuses are filled with lymph and are part of the sinus system of the lymph node.[37]

Cervical lymph nodes contain blood vessels. The main artery enters the lymph node at the hilus, which then branches into arterioles. Some of the arterioles supply the capillary bed in the medulla and some of them run along the medullary trabeculae to the

cortex where the arterioles further branch into capillaries and supply the lymphoid follicles. The rest of the arterioles run along the trabeculae and reach the capsule where they anastomose with other branches.[57]

The venous system has a similar route as the arterial system. The venules converge to form small veins in the cortex. The small veins run along the trabeculae of the lymph node and reach the medulla where they further converge to form the main vein. The main vein leaves the lymph node at the hilus.[21,57,37]



Classification of lymph nodes

There are about 800 lymph nodes in the body and about 300 lymph nodes are located in the neck.[66] The American Joint

Committee on Cancer (AJCC, 1997) classification was developed to provide a simple and efficient way to classify the cervical lymph nodes and this classification is widely used by surgeons and oncologists.

The AJCC classification divides palpable cervical lymph nodes into seven levels which are based on the extent and level of cervical nodal involvement by metastatic tumour.[15]

Level I contains the sub-mental and sub-mandibular triangles bounded by the posterior belly of the digastric muscle, the hyoid bone inferiorly, and the body of the mandible superiorly.

Level II -contains the upper jugular lymph nodes and extends from the level of the skull base superiorly to the hyoid bone inferiorly.

Level-III -contains the middle jugular lymph nodes from the hyoid bone superiorly to the cricothyroid membrane inferiorly.

Level-IV- contains the lower jugular lymph nodes from the cricothyroid membrane superiorly to the clavicle inferiorly.

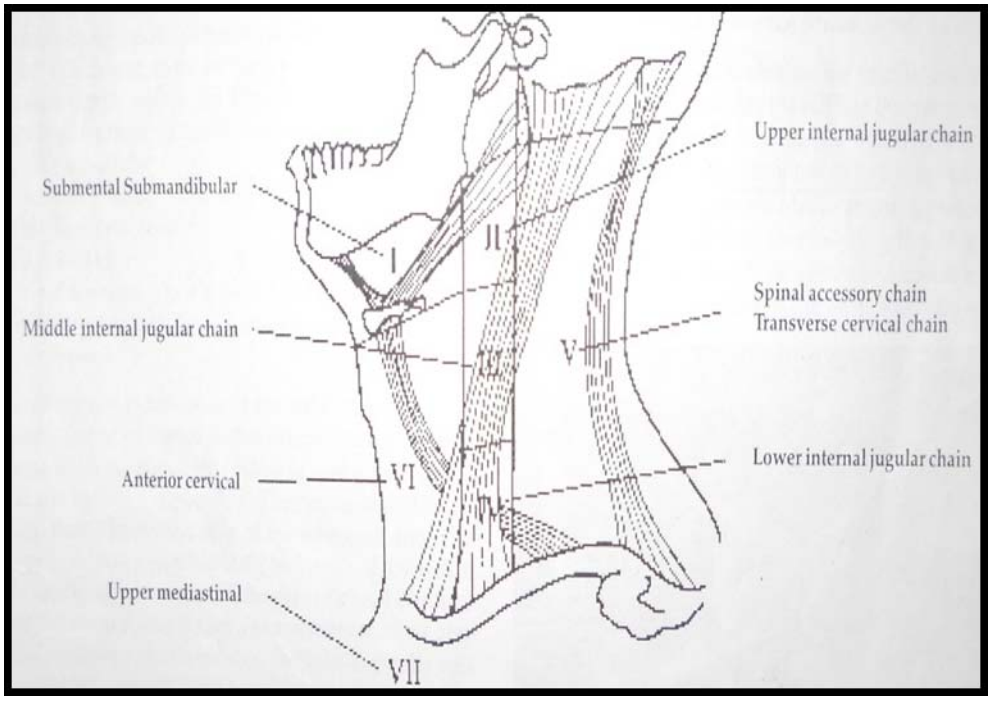
Level-V -contains the lymph nodes in the posterior triangle bounded by the anterior border of the sternocleidomastoid muscle anteriorly and the clavicle inferiorly. For descriptive purposes, level V may be further subdivided into upper, middle, and lower levels

corresponding to the superior and inferior planes that define levels II, III, and IV.

Level-VI -contains the lymph nodes of the anterior compartment from the hyoid bone superiorly to the suprasternal notch inferiorly. They lie between the medial borders of the carotid sheaths.

Level-VII contains the lymph nodes inferior to the suprasternal notch in the upper mediastinum.

Retropharyngeal, parotid, facial, occipital, and other nodes are referred to by these names.



Since the AJCC classification is used in different imaging modalities such as computed tomography and magnetic resonance imaging, some lymph nodes in this classification may be difficult to

be assessed by ultrasound, such as the paratracheal, prelaryngeal and upper mediastinal nodes.

In order to simplify the ultrasound examination of the neck and to ensure that all areas of the neck are covered in a systematic way, Hajek *et al* [36] developed another classification for ultrasound examination of the neck which is based on the location of the lymph nodes.

Region-1 includes lymph nodes located in sub-mental region.

Region-2 lymph nodes located in submandibular region.

Region-3 lymph nodes located in parotid region.

Region-4 lymph nodes located in upper cervical region.

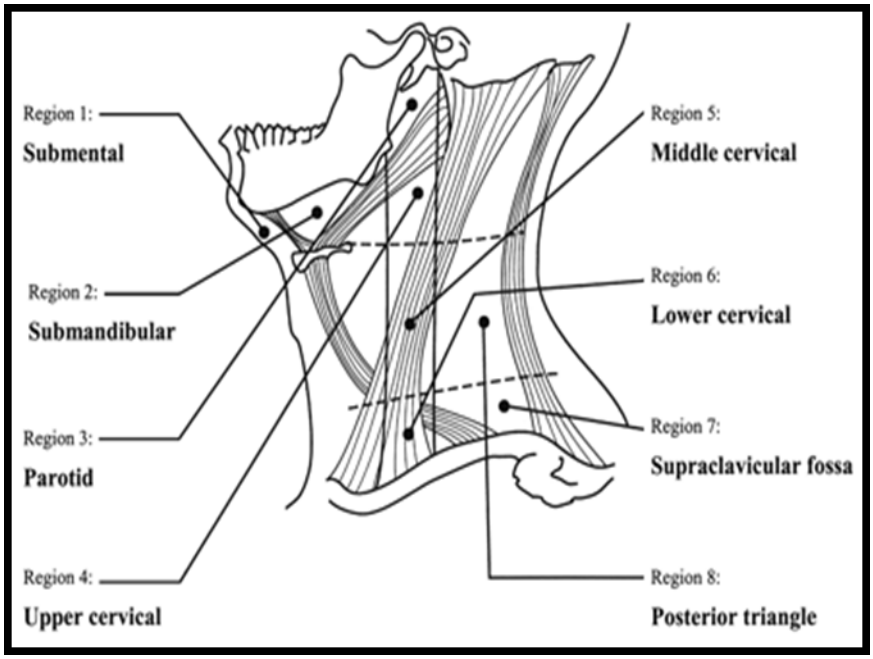
Region-5 includes middle cervical nodes.

Region-6 includes lower cervical nodes.

Region-7 nodes located in supraclavicular fossa.

Region-8 includes nodes in posterior triangle.

However, this classification is used to facilitate the ultrasound examination of the neck and should not be used for staging of carcinomas which is based on the AJCC classification.



PRINCIPLE OF COLOUR DOPPLER SONOGRAPHY

Doppler effect is a change in the observed frequency of wave because of motion of the source or observer.[48] Ultrasound images of flow are essentially obtained from measurements of movement. In ultrasound scanners, a series of pulses is transmitted to detect movement of blood. Echoes from stationary tissue are the same from pulse to pulse. Echoes from moving scatterers exhibit slight differences in the time for the signal to be returned to the receiver. These differences can be measured as a direct time difference or, more usually, in terms of a phase shift from which the ‘Doppler

frequency' is obtained. They are then processed to produce either a colour flow display or a Doppler sonogram.

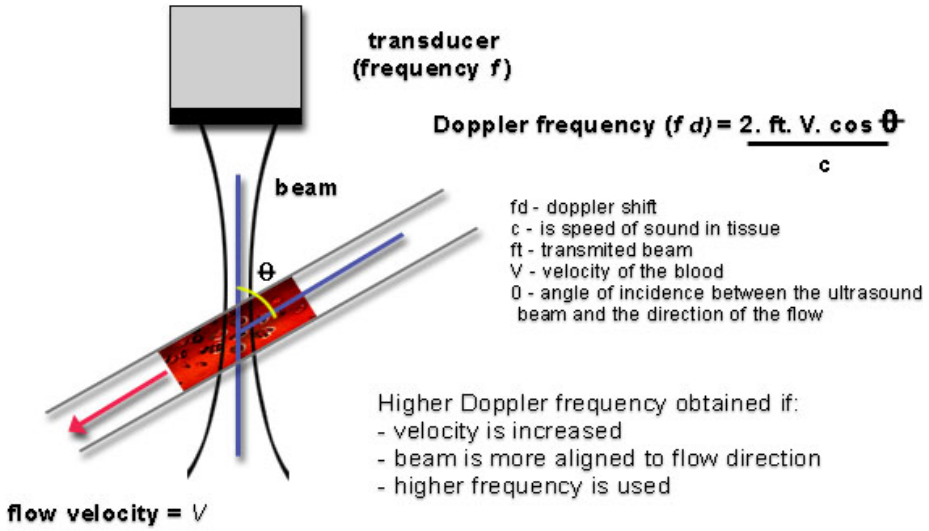


Figure 1: Doppler ultrasound. Doppler ultrasound measures the movement of the scatterers through the beam as a phase change in the received signal. The resulting Doppler frequency can be used to measure velocity if the beam/flow angle is known.

The size of the Doppler signal is dependent on *Blood velocity* (as velocity increases, so does the Doppler frequency); *ultrasound frequency* (higher ultrasound frequencies give increased Doppler frequency) the choice of frequency is a compromise between better sensitivity to flow or better penetration; *the angle of insonation* (the Doppler frequency increases as the Doppler Ultrasound beam becomes more aligned to the flow direction).

All types of Doppler ultrasound equipment employ filters to cut out the high amplitude, low-frequency Doppler signals resulting from tissue movement, for instance due to vessel wall motion.

CONTINUOUS WAVE AND PULSED WAVE:

As the name suggests, continuous wave systems use continuous transmission and reception of ultrasound. Continuous wave Doppler ultrasound is unable to determine the specific location of velocities within the beam and cannot be used to produce colour flow images.

Continuous wave Doppler is used in adult cardiac scanners to investigate the high velocities in the aorta.Doppler ultrasound in general and obstetric ultrasound scanners uses pulsed wave ultrasound. This allows measurement of the depth (or range) of the flow site. Additionally, the size of the sample volume (or range gate) can be changed. Pulsed wave ultrasound is used to provide data for Doppler sonograms and color flow images.

Aliasing: When pulses are transmitted at a given sampling frequency (known as the pulse repetition frequency), the maximum Doppler frequency **(fd)** that can be measured unambiguously is half the pulse repetition frequency. If the blood velocity and beam/flow angle being measured combine to give an **fd** value greater than half of the pulse repetition frequency, ambiguity in the Doppler signal occurs. This ambiguity is known as ***aliasing***.

The pulse repetition frequency is itself constrained by the range of the sample volume. The time interval between sampling

pulses must be sufficient for a pulse to make the return journey from the transducer to the reflector and back. If a second pulse is sent before the first is received, the receiver cannot discriminate between the reflected signal from both pulses and ambiguity in the range of the sample volume ensues.

As the depth of investigation increases, the journey time of the pulse to and from the reflector is increased, reducing the pulse repetition frequency for unambiguous ranging. The result is that the maximum **fd** measurable decreases with depth. Low pulse repetition frequencies are employed to examine low velocities (e.g. venous flow). The longer interval between pulses allows the scanner a better chance of identifying slow flow.

Aliasing will occur if low pulse repetition frequencies or velocity scales are used and high velocities are encountered. Conversely, if a high pulse repetition frequency is used to examine high velocities; low velocities may not be identified. Since colour flow imaging provides a limited amount of information over a large region, and spectral Doppler provides more detailed information about a small region, the two modes are complementary and, in practice, are used as such.

Colour flow imaging can be used to identify vessels requiring examination, to identify the presence and direction of flow, to highlight gross circulation anomalies, throughout the entire colour flow image, and to provide beam/vessel angle correction for velocity measurements.

Pulsed wave Doppler is used to provide analysis of the flow at specific sites in the vessel under investigation. When using colour flow imaging with pulsed wave Doppler, the colour flow/B-mode image is frozen while the pulsed wave Doppler is activated. Recently, some manufacturers have produced concurrent colour flow imaging and pulsed wave Doppler, sometimes referred to as *triplex* scanning.

COLOUR FLOW IMAGING:

Colour flow Doppler ultrasound produces a colour-coded map of Doppler shifts superimposed onto a B-mode ultrasound image (**Colour Flow Maps**). Although colour flow imaging uses pulsed wave ultrasound, its processing differs from that used to provide the Doppler sonogram.

Colour flow imaging may have to produce several thousand colour points of flow information for each frame superimposed on the B-mode image. Colour flow imaging uses fewer, shorter pulses along

each colour scan line of the image to give a mean frequency shift and a variance at each small area of measurement. This frequency shift is displayed as a colour pixel. The scanner then repeats this for several lines to build up the colour image, which is superimposed onto the B-mode image. The transducer elements are switched rapidly between B-mode and colour flow imaging to give an impression of a combined simultaneous image.

The pulses used for colour flow imaging are typically three to four times longer than those for the B-mode image, with a corresponding loss of axial resolution. Assignment of colour to frequency shifts is usually based on direction (for example, red for Doppler shifts towards the ultrasound beam and blue for shifts away from it) and magnitude (different colour hues or lighter saturation for higher frequency shifts).

The colour Doppler image is dependent on general Doppler factors, particularly the need for a good beam/flow angle. Curvilinear and phased array transducers have a radiating pattern of ultrasound beams that can produce complex colour flow images, depending on the orientation of the arteries and veins.

Factors affecting colour flow image includes *Power* (transmitted power into tissue),*Gain*(overall sensitivity to flow

signals)*Frequency,* **Pulse repetition frequency also called scale(**high pulse repetition frequency reduces aliasing),***Area of investigation***(** larger area reduces frame rate), ***Focus*** **(**colour flow image optimized at focal zone),*Triplex colour* (pulse repetition frequency and frame rate reduced by need for B-mode/spectral pulses), *Persistence* (high persistence produces smoother image but reduces temporal resolution), *Pre-processing* (trades resolution against frame rate), *Filter* (high filter cuts out more noise but also more of flow signal), *Post-processing* (assigns colour map/variance).

SPECTRAL OR PULSED WAVE DOPPLER:

Pulsed wave Doppler ultrasound is used to provide a sonogram of the artery or vein under investigation. The sonogram provides a measure of the changing velocity throughout the cardiac cycle and the distribution of velocities in the sample volume (or gate) .If an accurate angle correction is made, then absolute velocities can be measured.

The best resolution of the sonogram occurs when the B-mode image and colour image are frozen, allowing all the time to be employed for spectral Doppler. If concurrent imaging is used (real-time duplex or triplex imaging), the temporal resolution of the sonogram is compromised.

Factors' affecting the spectral image includes:

***Gait* (sharpness of resolution),** *Filter* (high filter cuts out more noise but more of flow signal), *Post-processing* (assigns brightness to output), *Power* (transmitted power into tissue), *Gain* (overall sensitivity to flow signals), *Pulse repetition frequency* also called scale (low pulse repetition frequency to look at low velocities, high pulse repetition frequency reduces aliasing), *Gate size* (Beam steering can allow improved beam/flow angle for better accuracy of velocity calculation).

Calculation of absolute flow:

Total flow measurement using color or duplex Doppler ultrasound is fraught with difficulties, even under ideal conditions.[62] Errors that may arise include: (1) Those due to inaccurate measurement of vessel cross-sectional area, for example the cross-sectional area of arteries which pulsate during the cardiac cycle, (2) Those originating in the derivation of velocity.

These errors become particularly large when flow calculations are made in small vessels; errors in measurement of diameter are magnified when the diameter is used to derive cross-sectional area. As with velocity measurements, it is prudent to be aware of possible errors and to conduct repeatability tests.

Flow waveform analysis:

Non-dimensional analysis of the flow waveform shape and spectrum has proved to be a useful technique in the investigation of many vascular beds. It has the advantage that derived indices are independent of the beam/flow angle. Many different indices have been used to describe the shape of flow waveforms.[32]

All are designed to describe the waveform in a quantitative way, usually as a guide to some kind of classification.

Commonly used indices available on most commercial scanners are:

1. **Resistance index (RI)** (also called resistive index or Pourcelot's index)
2. **Systolic/diastolic (S/D) ratio**, sometimes called the A/B ratio
3. **Pulsatility index** (PI)

These indices are all based on the maximum Doppler shift waveform. The PI takes slightly longer to calculate than the RI or S/D ratio because of the need to measure the mean height of the waveform. It does, however, give a broader range of values, for instance in describing a range of waveform shapes when there is no end-diastolic flow.

In addition to these indices, the flow waveform may be described or categorized by the presence or absence of a particular feature, for example the absence of end-diastolic flow and the presence of a post-systolic notch. Generally, a low Pulsatility waveform is indicative of low distal resistance and high Pulsatility waveforms occur in high-resistance vascular beds, although the presence of proximal stenosis, vascular steal or arterio-venous fistulas can modify waveform shape.

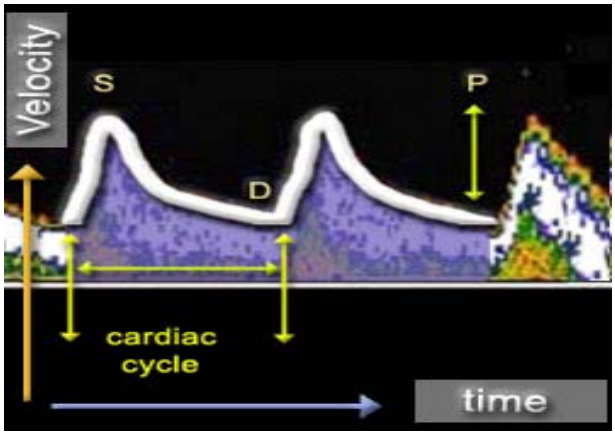


Figure 2: Arterial velocity sonogram (waveform).

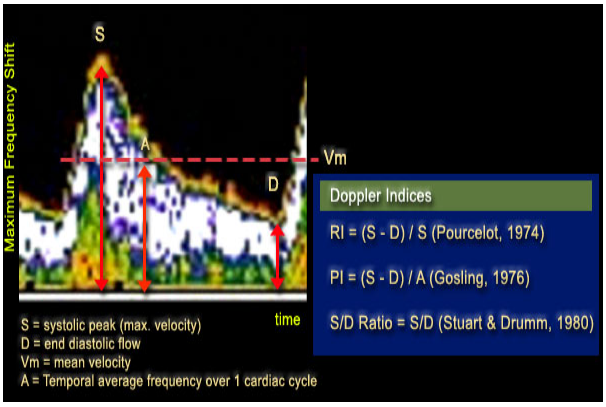


Figure 3 - Flow velocity indices

Care should be taken when trying to interpret indices as absolute measurements of either upstream or downstream factors. For example, alterations in heart rate can alter the flow waveform shape and cause significant changes in the value of indices.

Potential use of CDUS in differentiating metastatic from reactive lymph nodes

The vascular pattern of the enlarged lymph nodes is classified into the following four groups, according to the location of the vascularity[29]:

1. **Central**: flow signals branching radially from the centre, and signals are not along the periphery of the nodes.(Figure-1a)
2. **Peripheral**: flow signals along the periphery of the lymph nodes, with branches perforating the periphery of the node and not arising from the hilar vessels. (Figure-1b)
3. **Mixed**: The presence of central and peripheral flow signals. (Figure-1c)
4. **No flow**: absence of vascular signals within the lymph nodes. The presence of blood flow signals in the centre of node suggests that the node is reactive. The presence of peripheral, mixed or no flow suggests a metastatic nature.(Figure-1d)

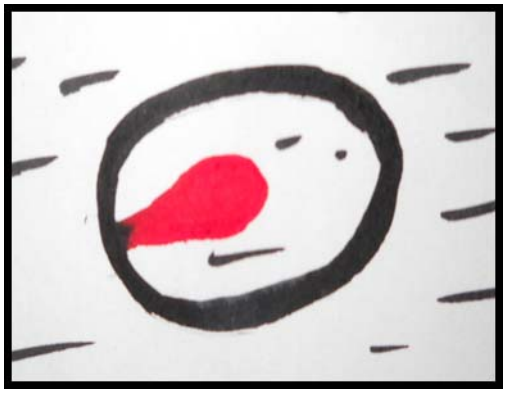


Figure 1a.Central flow

Figure 1b.Peripheral flow

Figure 1c.Mixed flow

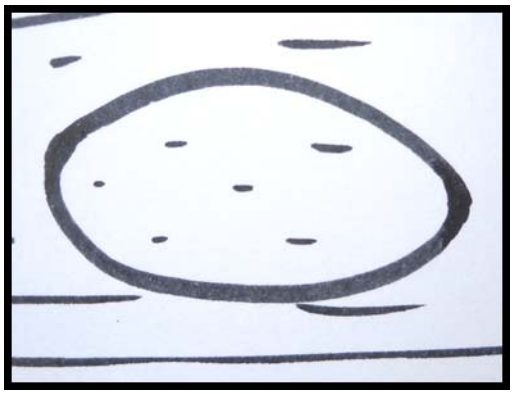


Figure 1d.No flow

On-board software is used for the evaluation of the vascular resistance, resistive index (RI) and Pulsatility index (PI), peak systolic velocity and end diastolic velocity of lymph nodes.

$$\text{Resistivity Index} = \frac{\text{Peak Systolic Velocity-End Diastolic Velocity}}{\text{Peak Systolic Velocity}}$$

$$\text{Pulsatility Index} = \frac{\text{Peak Systolic Velocity-End Diastolic Velocity}}{\text{Time-Averaged Maximum Velocity}}$$

The presence of high intranodal vascular resistance has been used as a key feature to differentiate reactive from metastatic nodes.

Metastatic nodes will have high PI and RI values as compared to low PI and RI values for Reactive nodes.

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC)

Aspiration of lymph nodes for diagnostic purposes was first done by Griey and Grayin 1904, in patients with sleeping sickness.[26] The experiment of fine needle aspiration (FNA) developed gradually, until 1921, when Guthrie[35] tried to correlate FNA results with various disease processes. FNAC is in practice since the 1930s.

The use of fine needle aspiration cytology (FNAC) in the investigation of lymphadenopathy has become an acceptable and widely practised minimally invasive technique, which is safe, simple, rapid and relatively pain-free.

FNAC is highly cost effective and accurate as a first line investigative technique with differential diagnoses including reactive hyperplasia/inflammatory conditions, granulomatous disorders and malignancy, stratifying cases requiring further investigations, surgical intervention or clinical follow-up.[44]

The main benefit of FNAC is to avoid the need for surgical biopsy, which requires local or general anaesthesia, increased hospital stay and costs.[60] Other advantages include It is economical, has better patient compliance, highly suitable for debilitated patient,

readily repeatable and is useful for multiple lesions.[56,59] The results are extremely satisfactory in good hands. The exact cytological diagnosis is available before definitive surgery is planned. It is cost contained procedure. It can relieve the anxiety and uncertainty in both patient and physician by making an accurate diagnosis preoperatively.

Diagnostic accuracy for metastatic lymph nodes approaches 97.9%, with sensitivity of 97.9% & specificity of 100%.[41] similar findings were observed by Kim et al who found them to be 97.9%, 97.9%, 99.1% respectively.[42]

Various studies conducted by Lee et al[45] and Nada[51] al Alwan et al also had somewhat similar result.

Ultrasound guided Fine Needle Aspiration Cytology can further be useful in small or clinically non-palpable nodes where chances of aspirating sufficient material for microscopic examination are less. In a resource challenged environment like ours, FNAC still remains the most acceptable, cheap and easily accessible modality for the diagnosis of metastatic lymphadenopathy.

Criteria used for diagnosing Reactive node by FNAC:[56]

1. A mixed population of lymphoid cells.
2. Numerical predominance of small lymphocytes.

3. Centroblasts, centrocytes, immunoblasts and plasma cells in variable but ‘logical’ proportions.
4. Dendritic reticulum cells associated with centroblasts and centrocytes (derived from germinal centres).
5. Scattered histiocytes with intracytoplasmic nuclear debris (tangible body macrophages).
6. Pale histiocytes, interdigitating cells, endothelial cells, eosinophils, neutrophils (variable).

Criteria used for diagnosing Metastatic node by FNAC[56]:

1. Abnormal nonlymphoid cells amongst normal/reactive lymphoid cells.
2. Cytological criteria of malignancy.

AIMS AND OBJECTIVES

1. To assess the diagnostic accuracy of CDUS in differentiating metastatic from reactive lymph nodes in oral cancer patients.
2. To evaluate the status of clinically not-palpable lymph nodes.
3. To correlate these findings with and Fine needle aspiration cytology (FNAC) findings.

REVIEW OF LITERATURE

R. A. Mountford et al (1979)[49] examined pathologically enlarged lymph nodes with a commercially available 10 MHz continuous-wave Doppler flow meter. Many enlarged lymph nodes gave rise to significant Doppler-shift signals indicating increased blood flow. The signals have been spectrum analysed and the large diastolic flow components suggest that there is considerable arterio-venous shunting within lymph glands involved in leukaemia; lymphoma and carcinoma. They suggested that these findings might be applicable to the detection of neoplastic tissues in less accessible sites.

Dun-Bing Chang et al (1994)[22] evaluated the efficacy of CDUS in detecting possible differences in blood flow pattern between malignant and benign cervical lymph nodes. They found sensitivity of 91% specificity of 63% of colour flow pattern alone in the diagnosis of malignant lymph nodes and sensitivity of 81% and specificity of 75% on the basis of intranodal vascular resistance.

Min Yun Choi et al (1995)[25] determined the value of Doppler spectral waveform to distinguish between benign and malignant lymph nodes. They found that lymph nodes involved by metastases showed a characteristic high RI(>1.0) and PI (>1.5) ,whereas lymph nodes involved by benign processes showed a low RI (<0.8) and low

PI (<1.5).They suggested that superficial lymphadenopathy due to benign and malignant diseases can be distinguished with a high degree of accuracy by means of spectral waveform analysis.

Dong Gyu Na et al (1997)[53] evaluated the usefulness of CDUS in differentiating benign from malignant cervical lymphadenopathy. Benign nodes showed central hilar vascularity in 94% of cases and abnormal flow pattern (absent or peripheral) was found in 98% of malignant and all tuberculous nodes. They established cut-off value of 0.8 for RI and 1.5 for PI that was 100% specific for malignancy. They concluded that CDUS combined with analysis of spectral waveform was useful in differentiating benign from malignant cervical lymphadenopathy.

Yoshiko Ariji et al (1998)[16] evaluated the usefulness of Power Doppler sonography in differentiating metastatic from non-metastatic cervical lymph nodes in patients with head and neck cancer. They concluded that the power Doppler criteria of no hilar flow, peripheral parenchymal nodal flow, and a transverse to longitudinal ratio of more than 0.65 together constitute a powerful tool for depicting metastatic lymph nodes in patients with cancer.

H-J Steinkamp et al (1998)[68] evaluated reactive as well as metastatic and malignant cervical lymph nodes by CDUS with

quantification of intensity of the perfusion. Reactive nodes showed intense hilar perfusion, metastatic nodes showed mainly peripheral flow and malignant node due to lymphoma showed intense hilar as well as peripheral flow. They concluded peripheral pattern may provide additional information in the differential diagnosis of cervical lymphadenopathy.

Zehra Hilal Adibellia et al (1998)[1] evaluated the efficacy of CDUS B-mode ultrasonography to differentiate between benign and malignant cervical lymph nodes. They concluded that both are of limited value in differentiating benign from malignant nodes .and cannot replace biopsy.

Chih-Hsiu Wu et al (1998)[72] used 2-D & 3-D PDUS in classifying the vascular patterns in cervical lymphadenopathy .they found that benign lymph nodes had a vascular (89%) and hilar (83%) flow, whereas malignant modes had spotted (72%), peripheral (60%), and mixed type (80%) of vascular patterns.(B-26-459002)

Anil T.Ahuja et al (1999)[13] studied colour duplex sonographic changes in successfully treated metastatic nodes from nasopharyngeal carcinoma in fourteen patients. The features studied included distribution of intra nodal vascularity, resistive and Pulsatility indexes, and peak systolic velocity. The majority (90%) of malignant

nodes from nasopharyngeal carcinoma have an increased central and peripheral vascularity, a high resistive index (0.8), and a high Pulsatility index (1.8). After radiation therapy to the nodes, a reduction in intranodal vascularity and a statistically significant reduction in the resistive index (0.58 to 0.59) and Pulsatility index (0.91 to 0.93) are found. They suggested that after radiation therapy for malignant nodes in nasopharyngeal carcinoma, a reduction in intranodal vascularity is found, and the resistive and Pulsatility indexes may return to benign parameters as early as 8 weeks after completion of treatment.

Chiung-Nien Chen et al (2000)[23] investigated the clinical usefulness of the colour Doppler vascularity index (CDVI) in patients with colon cancer before surgery. Forty-four patients with sonographically visible tumour mass of colon cancer were investigated. The CDVI was significantly higher in the patients with lymph node metastases and vascular invasion than in those without such metastases and invasion (P=0.006 and P=0.0098, respectively).Moreover, in patients with a high CDVI (>15%) and positive vascular invasion, survival was significantly poorer than in those with low CDVI (<15%) and negative invasion (P=0.0037 and 0.0039, respectively). According to the mode of recurrence in 36 patients who underwent curative

resection, the frequency of the distant organ recurrence was significantly higher in the high CDVI group (40%) than in the low CDVI group (0%). The CDVI is a good preoperative indicator of recurrence and patient survival in colon cancer. Thus, the CDVI may be helpful in stratifying patients for adjuvant therapy**.**

Toru Chikui et al (2000)[24] determined the most predictive sonographic features in cervical lymph nodes in patients with head & neck cancer by analyzing grey scale and power Doppler sonograms. They found that although multivariate analysis did not indicate any significant contribution of colour flow criteria for predicting metastatic nodes, the colour flow criteria appeared to improve the overall diagnostic accuracy for the less experienced observer.

HO S.S.Y. et al (2000)[39] performed colour Doppler sonography of malignant lymph nodes in patient with NHL and nasopharyngeal carcinoma in 60 nodes in 34 patients. They found significant difference in Doppler waveform between malignant nodes affected by lymphoma or nasopharyngeal carcinoma, suggesting that further differentiation of malignant nodes may be possible.

Tse Yang et al, (2000)[75] documented differences in colour Doppler flow and gray-scale Ultrasonographic (US) features between benign and malignant axillary lymph nodes in women with primary breast

cancer. The longitudinal-transverse axis ratio and hilar status on colour Doppler flow and gray-scale US images were prospectively studied for each of 145 axillary nodes in 135 women (74 palpable nodes in 69 women, 71 non palpable nodes in 66 women) with primary breast cancer. They concluded that Colour Doppler flow and gray-scale US features applicable to the identification of disease in palpable axillary nodes in patients with breast cancer are not applicable to non palpable nodes.

Jörg D. Moritz et al (2000)[48] determined whether contrast-enhanced colour Doppler sonography can differentiate benign from malignant enlarged cervical lymph nodes in head and neck tumours. Ninety-four enlarged lymph nodes in 39 adult patients were examined. With contrast-enhanced colour Doppler sonography, characteristic configurations were identified: hilar vessels with branching indicated lymphadenitis and predominantly peripheral vessels indicated metastases.

They concluded that enlarged lymph nodes can be characterized as metastatic or inflammatory with high diagnostic accuracy on the basis of their vascular architecture as seen on contrast-enhanced colour Doppler sonography.

Hiroshi Yusa et al (2000)[81] studied the usefulness of grey scale and CDUS for assessment for response to radio chemotherapy of metastatic cervical lymph nodes in head & neck cancer. Complete response nodes demonstrated hypo echoic internal echo and internal blood perfusion before radio chemotherapy. Most poor response nodes showed peripheral blood perfusion and avascular pattern. Complete response nodes showed a significant reduction in their maximum and minimum diameter.

Anil T. Ahuja et al (2001)[5] identified the parameters used in Doppler sonography in differentiating benign from metastatic neck nodes. They found that metastatic nodes tend to have higher intranodal vascular resistance than reactive nodes, but there was considerable overlap of resistance parameter between two groups. Metastatic nodes showed peripheral vascularity; majority of reactive nodes showed hilar vascularity. They concluded that the distribution of intranodal vascularity appears to be more useful than RI or PI in differentiating benign and from malignant cervical lymphadenopathy.

Anil Ahuja et al (2001)[6] evaluated the tuberculous lymphadenitis and metastatic nodes from nasopharyngeal carcinoma by power Doppler sonography. The intranodal vascular appearances of

tuberculous neck nodes were compared with benign reactive neck nodes and metastatic nodes from nasopharyngeal carcinoma.

They found that the intranodal vascular distribution in tuberculous nodes was varied and simulated both benign and malignant disease. Avascularity of nodes and displacement of hilar vascularity were frequent in tuberculous nodes. They also found that Metastatic nodes from nasopharyngeal carcinoma had a higher vascular resistance than did tuberculous nodes and tuberculous nodes had a higher vascular resistance than did reactive nodes.

Anil Ahuja et al (2001)[11] investigated the difference in the nodal hilus evaluated by gray scale and power Doppler sonography. They included One hundred ninety-two patients with proven cervical lymphadenopathy in the study (metastases, n = 118; tuberculosis, n = 56; and lymphoma, n = 18). Lymph nodes were evaluated by gray scale sonography for the echogenic hilus and power Doppler sonography for hilar vascularity. They found hilar vascularity even though the lymph node did not show an echogenic hilus on gray scale sonography. They concluded that gray scale and Doppler sonography show different aspects of the hilus, and the absence of the hilus on gray scale sonography does not necessarily imply an associated absence of hilar vascularity.

Takako Shirakawa et al (2001)[65] used colour/power Doppler sonography in differential diagnosis of superficial lymphadenopathy. They found extra hilar vessels in 85.4% of metastatic, 40.5% of lymphomatous and 7.7% of benign nodes regardless of node size. The mean RI & PI values of metastatic nodes were higher than those of benign nodes with lymphomatous nodes had intermediate values. They concluded that PI >1.3 & RI>0.72 suggested malignancy as measured from any vessel.

Misa Sumi et al (2001)[69] compared the ability of sonography and CT to differentiate benign from malignant cervical lymph nodes by analyzing 209 cervical nodes (102 metastatic and 107 non metastatic) from 62 patients with head and neck cancer. They concluded that sonography performed significantly better than CT in depicting cervical metastatic nodes.

Koichi Yonetsu et al (2001)[80] tested whether the combined use of size criteria and Doppler Sonographic findings would improve the predictive ability for metastatic cervical nodes. They analyzed 338 histologically proved cervical lymph nodes (108 metastatic and 230 non metastatic) in 73 patients with head and neck cancer. The combined criteria yielded high sensitivities (89%) and specificities (94%).They concluded that hilar blood flow information obtained by

Doppler sonography significantly improves diagnostic accuracy for the detection of nodes metastatic from head and neck squamous cell carcinoma.

Tatsuya Sakaguchi et al (2001)[62] differentiated reactive small round lymph nodes (SRLNs) from metastases by power Doppler ultrasonography (PD-US) and contrast-enhanced CT (CE-CT) performed in 99 cervical lymph nodes (LNs) with a maximum diameter of 1.5 cm or smaller and maximum longitudinal/transverse ratio of 1.5 or smaller in 76 patients with head and neck cancer. They concluded vascular patterns evaluated with PD-US and enhancement patterns on CE-CT can characterize SRLNs. For an avascular pattern on PD-US, information on CE-CT results can significantly increase the accuracy of characterization.

Francesco Giovagnorio et al (2002)[34] performed a retrospective study to document the sonographic and colour Doppler characteristics of lymphomatous superficial lymph nodes in 130 individuals who underwent sonography, colour Doppler imaging, fine-needle aspiration biopsy, and surgical removal of the nodes with the final diagnosis of lymphoma (87) and chronic adenitis (43). They concluded that the presence of peripheral sub capsular vessels, which is typical of metastasis, is definitely rare in lymphoma (with the

possible exception of the uncommon subtypes of high-grade lymphomas).

Ying et al (2002)[76] investigated the racial difference in gray scale and power Doppler sonography of cervical lymph nodes between white and Chinese subjects. Lymph nodes were evaluated for their number, size, site, echogenic hilus, and vascular pattern, degree of vascularity, blood flow velocity, and vascular resistance in 20 healthy white and 20 healthy Chinese subjects. They found that there were no significant differences in the number, size, and distribution of the lymph nodes between the 2 populations. They also found that there was no significant difference in the gray scale and vascular features of cervical nodes between white and Chinese subjects. This study concluded that results on power Doppler and gray scale sonographic assessment of cervical Lymphadenopathy reported in previous studies may be applicable in both populations.

Monika-Hildegard Schmid-Wendtner et al (2002)[64] evaluated administration of a D-galactose–based signal enhancer in colour Doppler sonography (CDS) for better detection of vascularity patterns, to differentiate malignant from benign lymph nodes in patients with cutaneous melanomas with comparison of B-mode sonography, native CDS, and signal-enhanced CDS. They concluded

that administration of a D-galactose–based signal enhancer for CDS in patients with cutaneous melanomas can help to differentiate malignant from reactive lymph nodes, hematomas, or seromas.

Sato Eida et al (2003)[31] used a combination of contrast-enhanced helical CT and Doppler sonography to monitor the necks of 58 patients with initial clinical N0 stage neck disease. The combined criteria were effective in revealing 26 (87%) nodes, yielding 87% sensitivity, 100% specificity, and 100% positive and 99% negative predictive values. The independent use of one of these techniques alone resulted in low (67%) or moderate (87%) positive predictive values for sonography and CT, respectively. They concluded that the combination of contrast-enhanced helical CT and Doppler sonography is useful for the follow-up study of clinical N0 stage neck disease.

Gernot Schulte-Altedorneburga et al (2003)[14] used contrast agent with CDUS & PDUS to improve their diagnostic accuracy in differentiating benign and malignant superficial lymph node enlargement. They concluded that use of contrast agent seems not to improve the diagnostic accuracy of CDUS & PDUS compared with native colour mode studies.

Michael Ying et al (2004)[79] evaluated the accuracy of power Doppler Sonographic features in differentiating cervical lymphadenopathy in different diseases, in 270 patients by using FNAC or Excisional biopsy for confirmation. They found that vascular pattern was more useful in differentiating reactive from malignant nodes with a sensitivity of 88% and specificity of 100%.RI>0.8 &PI > 1.5 was more accurate for metastatic nodes with a accuracy of 65% & 77% respectively.

Anil T. Ahuja et al (2004)[10] compared the performance of colour Doppler, power Doppler and 3-D power Doppler sonography in the assessment of vascular pattern and vessel displacement of cervical lymph nodes. Colour Doppler, power Doppler and 3-D power Doppler sonograms of 145 cervical nodes were reviewed (metastases *n* = 60, lymphoma *n* = 30, tuberculosis *n* = 23, reactive *n* = 25, Kimura disease *n* = 7). Results showed that there was a high level of agreement between CDS and PDS in assessment of vascular patterns, whereas the level of agreement between CDS and 3-D PDS and between PDS and 3-D PDS was low. There was a high level of agreement in the assessment of displacement of hilar vascularity among the three imaging modes. In the assessment of vascular patterns of cervical lymphadenopathy, CDS and PDS have a similar

performance. However, accurate assessment of the vascular pattern of cervical nodes may be difficult using 3-D PDS. In the assessment of displacement of hilar vascularity, the performances of CDS, PDS and 3-D PDS are similar.

Özerk ömür ökten (2004)[54] evaluated the value of the gray-scale, colour Doppler and power Doppler ultrasonography in the differential diagnosis of lymphomatous superficial lymph node enlargements. The study group consisted of 33 males and 16 female. During the CDUS and PDUS examination, they classified the nodes into 3 patterns: type I, “hilar normal”; type II, “hilar activated”; and type III, “peripheral”. Pulsatility (PI) and resistivity RI) indexes were calculated using CDUS. When clinical data and histopathological findings were combined, 27 subjects were diagnosed as lymphoma, and 22 cases were classified as lymphadenitis. Semi quantitative CDUS parameters are not valuable in the differential diagnosis of lymphomatous superficial lymph node pathologies. Qualitative GSUSG and PDUSG features, may be used as an alternative work-up to cytological studies in patients in whom diagnostic surgical procedures cannot be performed.

Huang Y et al (2008)[40] determined whether the resistive index (RI) ratio based on high-resolution spectral Doppler sonography would be

useful in the differential diagnosis of small inflammatory and metastatic lymph nodes in rabbit models. They evaluated the sizes, long-/short-axis ratios, and RI ratios (defined as the value of the peripheral RI relative to the central RI) of the nodes were evaluated with sonography and then compared with the histopathologic findings. They found no statistical differences were found between the volumes and the long-/short-axis ratios of 15 inflammatory and 14 metastatic lymph nodes. On spectral Doppler sonography, the RI ratio was higher in the metastatic lymph nodes than in the inflammatory lymph nodes. They concluded that RI ratio changes based on high-resolution spectral Doppler sonography are associated with histopathologic changes of metastatic and inflammatory lymph nodes during the initial stage in rabbits.

Anil Ahuja et al (2008)[12] evaluated different causes of lymph node enlargement by ultrasound. They noted the following observations. On ultrasound; grey scale sonography helps to evaluate nodal morphology, whilst power Doppler sonography is used to assess the vascular pattern. Grey scale sonographic features that help to identify metastatic and lymphomatous lymph nodes include size, shape and internal architecture (loss of hilar architecture, presence of intranodal necrosis and calcification). Soft tissue edema and nodal

matting are additional grey scale features seen in tuberculous nodes or in nodes that have been previously irradiated. Power Doppler sonography evaluates the vascular pattern of nodes and helps to identify the malignant nodes. In addition, serial monitoring of nodal size and vascularity are useful features in the assessment of treatment response.

Dangore et al (2008)[28] evaluated the efficacy of colour Doppler ultrasound (CDUS) to differentiate between benign and malignant cervical lymph nodes. CDUS was performed for 168 cervical lymph nodes in these 100 patients. Histopathological confirmations were obtained by fine needle aspiration biopsy and/or excisional biopsy. They compared the clinical features, CDUS features and cytological/histological features of enlarged cervical lymph nodes. Correlation of patterns of colour Doppler flow signals with pathological diagnosis showed that central flow for benign nodes and peripheral flow for malignant nodes were highly significant parameters. They found that CDUS has a higher specificity than clinical evaluation, being 94.28% and 58.76%, respectively. Accuracy of the CDUS examination was also definitely higher than clinical evaluation at 92.85% and 63.67%, respectively.

Suwarna Dangore-Khasbage et al (2009)[29] studied the utility of CDUS in evaluating the status of cervical lymph nodes in 30 oral cancer patients. They used intranodal vascular flow pattern and vascular resistance as the key CDUS features to differentiate between metastatic and reactive cervical lymph nodes. They found CDUS to be highly significant with a sensitivity of 92.90% and a specificity of 84.21% after comparing the CDUS findings with histopathological findings.

MATERIAL AND METHODS

This study was conducted in the Department of Oral Medicine and Radiology, Tamil Nadu Government dental college and hospital, Chennai-3; Barnard Institute of Radiology, Madras Medical College, Chennai-3; Goschen Institute of Pathology, Madras Medical College, Chennai-3.This study was carried out after obtaining approval from Institutional Ethical Committee and informed written consent from each patient. Study comprised 80 Adult Subjects with clinically and histopathologically diagnosed Oral cancer of either gender with age range of 20-60 years. All the findings were recorded in a structured Performa designed for the purpose of this study.

To avoid over-diagnosis of lymph nodes in patients with oral cancer, study was divided in two groups based on clinical criteria. Group-I included 40 patients with clinically suspected Metastatic cervical lymph nodes, Group-II included 40 patients with clinically suspected Reactive Cervical lymph nodes. Inclusion criteria were patients with oral cancer diagnosed on the basis of thorough history and clinical examination followed by histopathological confirmation.

Exclusion criteria were those Patients with acute cervical lymph node enlargement due to infections; with malignancies of lymph nodes like Lymphoma, lymphocytic leukaemia; with

generalized lymphadenopathy; in cases where FNAC was inconclusive.

The clinical stage and location of primary tumour was determined according to the recommendations of American Joint Committee on Cancer Staging.[15] (Figure-2, 3)

Initially, the cervical lymph nodes were assessed based on clinical criteria to differentiate metastatic lymph nodes from reactive nodes (Figure-4). Clinical criteria used for differentiating metastatic lymph nodes were (I) lymph node size larger than 1 cm in diameter, (ii) a hard, and stony hard in consistency, (iii) fixation to underlying structures.[33, 58] Based on these criteria 2 groups were formed: Group-I of 40 patients with clinically suspected Metastatic Cervical lymph nodes and Group-II of 40 patients of clinically suspected Reactive lymph nodes.

After getting the informed consent, the same patients with oral cancer were evaluated with Colour Doppler Ultrasound (CDUS) for cervical lymph node involvement (Figure-6). The CDUS was performed by an experienced radiologist by using Linear Multi-frequency Transducer with range of 5MHz-13MHz (ACUSON ANTARES MODEL PREMIUM EDITION, SIEMENES MEDICAL).The radiologist had not been given any clinical data or

pathological diagnosis in any of the cases and the findings were recorded in a tabular format. Initially adequate observation of the lymph node with grey scale sonography was done followed by colour Doppler evaluation.

Different criteria considered in CDUS of the lymph nodes were colour flow signals and intranodal vascular resistance (Pulsatility Index and Resistivity Index).

The vascular pattern of the enlarged lymph nodes was classified into the following four groups, according to the location of the vascularity:

1. **Peripheral** (Figure-7)
2. **Mixed** (Figure-8)
3. **No flow** (Figure-9)
4. **Central** (Figure-10)

The presence of blood flow signals in the centre of node suggests that the node is reactive. The presence of peripheral, mixed or no flow suggests a metastatic nature.

On-board software was used for the evaluation of the vascular resistance, Resistive index (RI) and Pulsatility index (PI), peak systolic velocity and end diastolic velocity of lymph nodes. The presence of high intranodal vascular resistance had been used as a

key feature to differentiate metastatic from reactive nodes. Metastatic nodes will have high PI and RI values as compared to low PI and RI values for Reactive nodes. Cut-off values used for PI was 1.5 and for RI was 0.7 as suggested by Wu et al.[73] PI and RI more than cut-off values suggested metastatic node whereas PI and RI less than cut-off suggested a reactive node.

Cytological diagnosis of the same lymph nodes was made from FNAC or ultrasound guided FNAC with patient in supine position. (Figure-12) Ultrasound guided FNAC was used to guide needle at proper location in the lymph node, in cases where unguided FNAC was not possible like in deep seated node, in obese patients due to thick overlying tissue. In our study 5 patients out of 80 patients underwent US guided FNAC.

FNAC was used as a standard, to confirm the etiology of the lymph node enlargement. Each node was cytologically determined to be metastatic or reactive. In patients with many lymph nodes belonging to the same group of lymph nodes with similar CDUS appearance, the most prominent and accessible node was subjected to FNAC and its cytopathological appearance was considered for correlation with CDUS features of all lymph nodes from the same group. In all oral cancer patients, FNAC was suggestive of either

Metastatic (Figure-13) or Reactive (Figure-14) lymph node involvement.

All aspirated material from lymph nodes were smeared on two slides. One slide was fixed in Isopropyl Alcohol (70%-90%) for 20-30 minutes followed by H & E staining; Second slide was fixed in 95.6% methanol for MGG (May-Grunewald-Giemsa solution) staining for more nuclear details. Each lymph node was evaluated pathologically with an emphasis on the presence or absence of dysplastic cells in cytology. An experienced pathologist performed the cytological evaluation of the cervical lymph nodes. A comparative study was done between the clinical features, CDUS and the cytological features of cervical lymph nodes. Chi-Square Test (SPSS 15.0 version) was used to evaluate the significance of the parameters used between the two study groups.

INSTRUMENTATION

Clinical examination Armamentarium (Figure-1)

- Disposable gloves
- Face mask
- Patient’s apron
- Stainless steel tray
- Mouth mirror, explorer and tweezer for each patient.

- Gauze
- Retractor
- Nikon Coolpix S3000 Digital camera (12 megapixels) to capture images.

Colour Doppler ultrasound examination Armamentarium (Figure-5)

- Ultrasound machine (Acuson Antares Premium Edition-Siemens Medical)
- VFX 13-5(Multi-D) Ultrasound Probe
- DIAMED Ultrasound Gel

Fine Needle Aspiration Cytology examination Armamentarium (Figure-11)

- 5 ml. syringe with 22 gauge needle for taking FNA samples.
- Glass Slides
- 70-90% Isopropyl Alcohol in Coplin Jar
- 95.6% Methanol in Coplin Jar
- H & E stain
- MGG stain

INSTITUTIONAL ETHICAL COMMITTEE
Tamil Nadu Government Dental College and Hospital, Chennai-3

Telephone No : 044 2534 0343
Fax : 044 2530 0681

R.C.No. 0431/DE/2010

Date: 01.06.2010

Title of the Work : Diagnostic Accuracy of Colour Doppler Ultrasonography in Evaluation of Cervical Lymph Nodes in Oral Cancer Patients.

Principal Investigator: Dr.Suresh Kumar, IIIrd Yr PG student

Department : Dept of Oral Medicine & Radiology
Tamil Nadu Govt Dental College and Hospital, Chennai-3

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 22.04.2010 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"Advised to Proceed with the study"

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the Principal Investigator.

The Principal Investigator and their team are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the Institution.
6. You should complete the work within the specific period and if any extension of time is required. You should apply for permission again and do the work.
7. You should submit the summary of the work to the ethical committee on completion of the work.
8. You should not claim funds from the Institution while doing the work or on completion.
9. You should understand that the members of IEC have the right to monitor the work with prior intimation.
10. Your work should be carried out under the direct supervision of your Guide/Professor.

S Jayachandran 01/6/10.
SECRETARY

01/6/10
CHAIRMAN

DECLARATION

TITLE OF DISSERTATION	**DIAGNOSTIC ACCURACY OF COLOUR DOPPLER ULTRASONOGRAPHY IN EVALUATION OF CERVICAL LYMPH NODES IN ORAL CANCER PATIENTS**
PLACE OF THE STUDY	Department of Oral Medicine and Radiology, Tamilnadu Government Dental College & Hospital,Chennai-03 Barnard Institute of Radiology, Govt.General Hospital & MMC, Chennai-03 Goschen Institute Of Pathology, Govt. General Hospital & MMC,Chennai-03
DURATION OF THE COURSE	3 YEARS
GUIDE & HEAD OF THE DEPARTMENT	**DR. S. JAYACHANDRAN**

I hereby declare that no part of dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Tamilnadu Government Dental College and Hospital, Chennai-03.In addition,I declare that no part of this work will be published either in print or in electronic media without the Guide, who has been actively involved in dissertation. The author has the right to preserve for publishing of the work solely with prior permission of the a Principal, Tamilnadu Government Dental College and Hospital, Chennai-03.

Guide & Head of the Department Signature of the candidate

INFORMED CONSENT FORM

DIAGNOSTIC ACCURACY OF COLOUR DOPPLER ULTRASONOGRAPHY IN EVALUATION OF CERVICAL LYMPH NODES IN ORAL CANCER PATIENTS

Name: O.P.No:

Address: Serial No:

Age / Sex:

Tel. no:

I, _____age____years
Exercising my free power of choice, hereby give my consent to be included as a participant in the study "Diagnostic Accuracy of Colour Doppler Ultrasonography in Evaluation of Cervical Lymph nodes in Oral cancer Patients."
I agree to the following:

1. I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
2. I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
3. I have informed the doctor about all medications I have taken in the recent past and those I am currently taking and other systemic illness that I have.
4. I agree to report to the doctor for a regular follow-up as and when required for the research.
5. I hereby give permission to use my medical records for research purpose.
6. I have been told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature Date

அறிவிக்கப்பட்ட ஒப்புதல் படிவம்

Diagnostic Accuracy of Colour Doppler Ultrasonography in Evaluation of Cervical Lymph Nodes in Oral Cancer Patients

பெயர்: மருத்துவ பதிவு எண்:

முகவரி: வரிசை எண்:

தொலைபேசி எண்: வயது:
ஆண்/பெண்

நான் ..... த/பெ .....

வயது .....

என்ற விலாசத்தில் வசிக்கின்றேன். நான் எனக்கு உள்ள சுதந்திரத்தின் மூலமும், சுய உரிமையின் மூலமும் "வாய்ப்புற்று நோயில் கழுத்துப்புகுதியில் உள்ள நெறிக் கட்டிகள்" குறித்த கலர் டாப்ளர் மற்றும் அல்ட்ரா சவுண்ட் இவற்றின் துல்லியம் பற்றிய பரிசோதனைக்கு என்னை உட்படுத்திக் கொள்ள அனுமதிக்கிறேன்.

நான் கீழ்கண்டவற்றிற்கு ஒப்புக் கொள்கிறேன்

1. எனக்கு செய்யப்படவுள்ள பரிசோதனை முறைகள் பரிசோதனையின் காரணம், மற்றும் என் உடல்நிலை பாதுகாப்பு, உடல் இயக்கம் பற்றிய பாதுகாப்பு முறைகள் எனக்கு தெளிவாகக் கூறப்பட்டுள்ளது.

2. நான் மருத்துவருக்கு முழுமையாக ஒத்துழைக்கவும் எனக்கு உடலில் ஏதேனும் பிரச்சனைகள், மாறுதல்கள் ஏற்பட்டால் மருத்துவருக்கு உடனே தெரியபடுத்தவும் ஒப்புக் கொள்கிறேன்.

3. நான் தற்போது உட்கொள்ளும் மருந்துகள், மாத்திரைகள், மற்றும் உடலில் உள்ள நோய்கள் குறித்து மருத்துவருக்கு முழு தகவல்களையும் அளித்துள்ளேன்.

4. ஏற்கனவே குறிப்பிட்டுள்ள நேரங்களிலும் தேவைப்படும் நேரங்களிலும் தவறாமல் வந்து மருத்துவரை சந்திக்க ஒப்புக் கொள்கிறேன்.

5. என் அடையாளத்தை ரகசியமாக வைத்துக் கொள்ள எனக்கு பரிசோதனை செய்யும் மருத்துவரும் மருத்துவமனையும் ஒப்புக் கொண்டுள்ளது.

6. இதன் மூலம் நான் என் மருத்துவக் குறிப்புகளை மருத்துவ ஆராய்ச்சிக்கு பயன்படுத்த ஒப்புக் கொள்கிறேன்.

ஒப்பம்/ இடது கை பெருவிரல் ரேகை

நோயாளியின் பெயர்/
பரிசோதனைக்கு உட்படுபவர் பெயர்

பரிசோதிப்பவர்/
மருத்துவர் பெயர்

நாள்:
இடம்:

DIAGNOSTIC ACCURACY OF COLOUR DOPPLER ULTRASONOGRAPHY IN EVALUATION OF CERVICAL LYMPH NODES IN ORAL CANCER PATIENTS

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CHENNAI – 600003

PROFORMA

Date: Dental O.P. No: Serial No:

Name: Age / Sex:

Address: Tel. no:

Occupation: Income:

Chief Complaints with Duration:

Past Medical history:

1. Epilepsy
2. Tuberculosis
3. Jaundice
4. Pregnancy / Lactation
5. Prolonged medication
6. Hypertension
7. Asthama
8. Diabetes Mellitus
9. Cardiac diseases
10. Others

Past Surgical History:

Dental history:

Personal History:

GENERAL EXAMINATION:

Nutritional Status: Good/ Moderate / Poor

Anemia: Loss of Weight:

HEAD AND NECK EXAMINATION:

Extra – Oral Examination:
CERVICAL LYMPH NODE EXAMINATION:

A.INSPECTION:

1. Site

2. Number: Size:

B. PALPATION:

1. Local Temperature: 2.Number

3. Tenderness:

4. Consistency: Firm/Hard/Stony Hard
Indurated or Variable

5. Fixity to Surrounding Structures:

Intra-Oral Examination:

Oral hygiene: Poor /Fair /Good
Mouth Opening:
Teeth: Mobile: Pain:
Lost: Irritation:
Difficulty in mastication:

Dysphagia: Loss of Taste:

Tongue Protrusion:

Numbness /Tingling:
Others:

Type of Lesion:

Nodular /Growth / Gangrenous / Ulcer / Ulcerated growth / Papillary / Induration

Fissured / Unicentric / Multicentric / Others

Extension:

Size:
Description:

Associated /Preexisting / Precancerous conditions / Lesion:

Leukoplakia Oral Sub mucous Fibrosis Lichen planus
Others:

RADIOGRAPHIC FINDINGS:
Bone Involvement: Erosion / Infiltration / Intra Bony / Pathological Fracture
Other:

DIAGNOSIS:

CLINICAL STAGING: T……N……M……..

INVESTIGATIONS:
1. Laboratory Investigations:

a. Blood : TC DC
Hb% ESR
Bleeding Time Clotting Time
Peripheral Smear:

b. Urine : Sugar :
Albumin:

2. Colour Doppler Ultrasound finding:

3. FNAC finding:

Signature:

Date:

Time:

PHOTOGRAPHS

CLINICAL EXAMINATION

Figure-1.Armamentarium

Figure-2 Case-1 Oral cancer involving Right buccal mucosa

Figure-3 Case-2 Oral cancer involving Left retromolar trigone

Figure-4.Evaluation of cervical node by clinical palpation

COLOUR DOPPLER ULTRASONOGRPHY

Figure-5 Colour Doppler Ultrasound Unit (Acuson Antares Premium Edition, Siemens Medical)

Figure-6.Evaluation of left submandibular lymph node in the oblique plane with the use of transducer

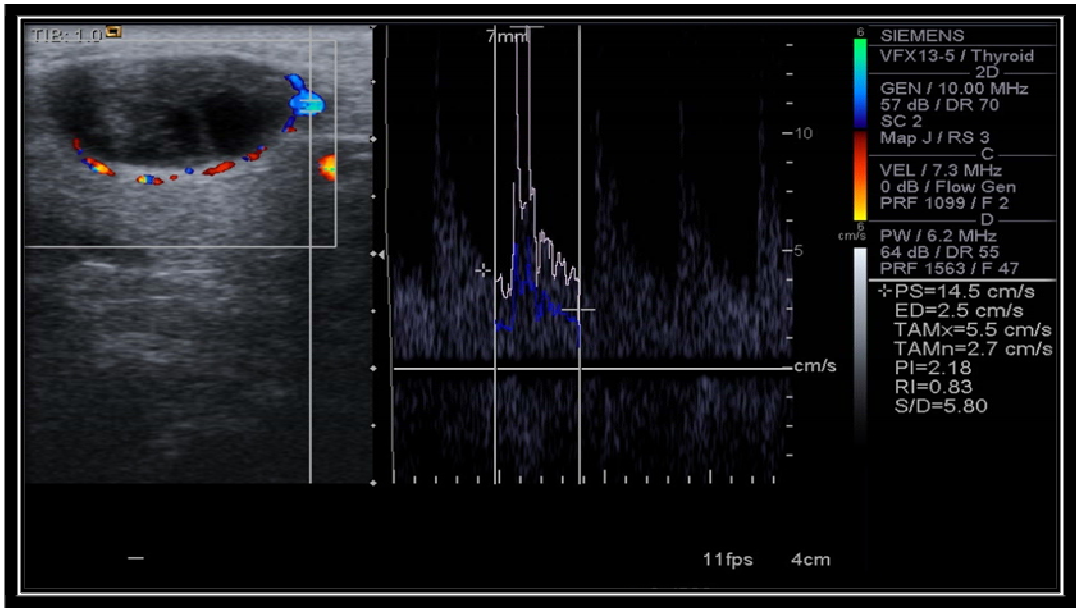


Figure-7.Metastatic Lymph node with Peripheral flow pattern (L) Spectral wave form pattern (C) and Vascular indices values (R) (Case-1)

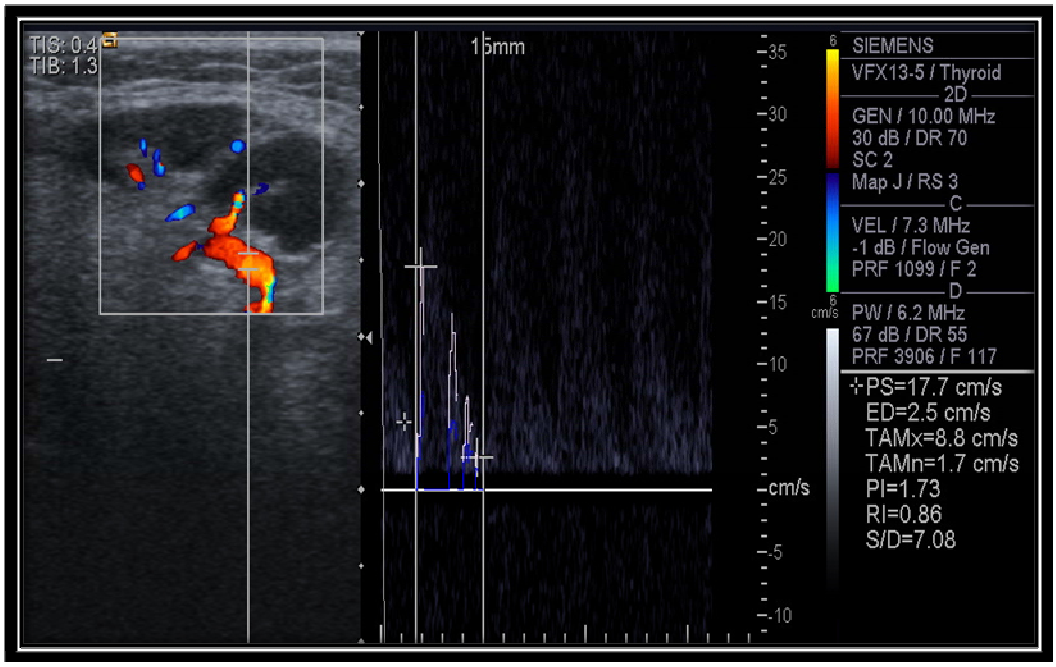


Figure-8.Metastatic Lymph node with Mixed flow pattern (L) Spectral wave form pattern (C) and Vascular indices values (R)

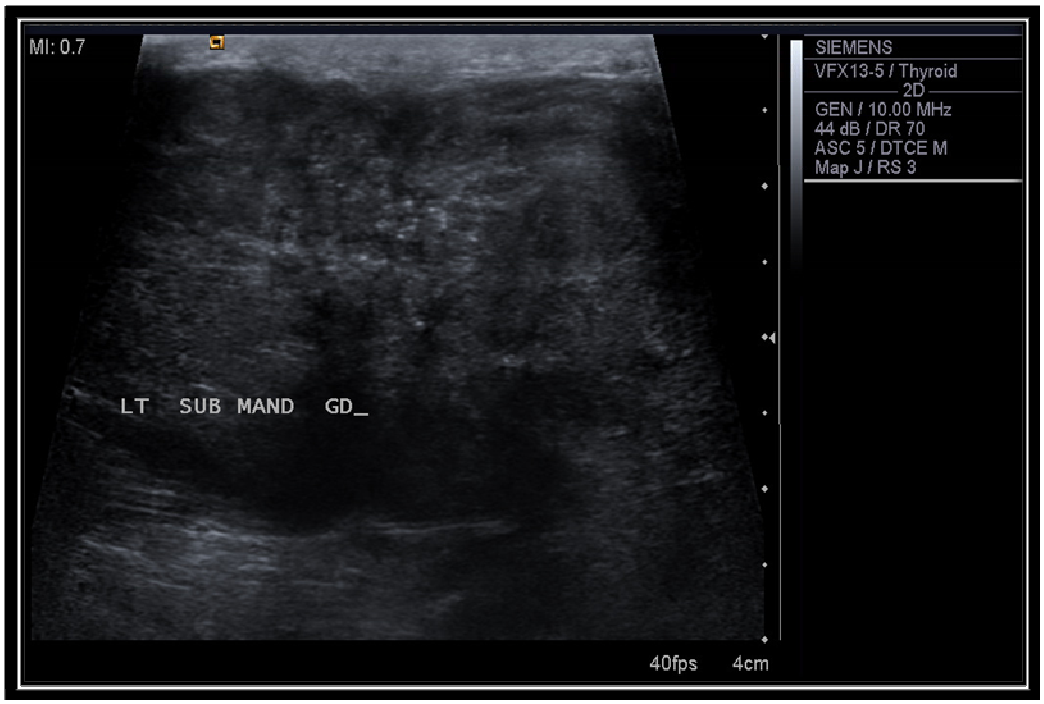


Figure-9.Metastatic Lymph node with No flow

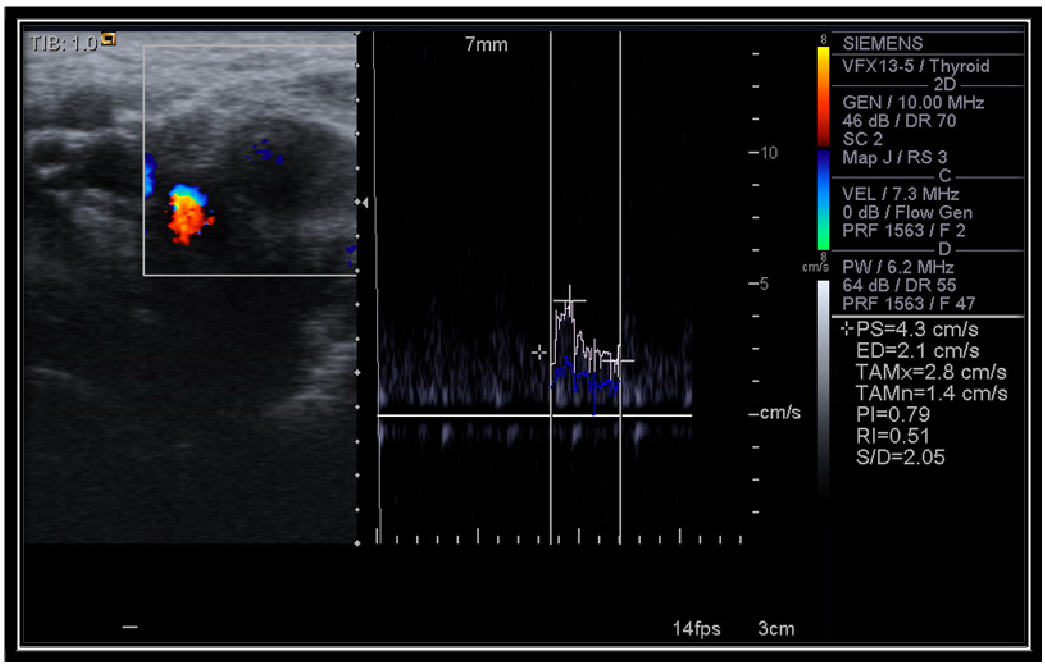


Figure-10.Reactive Lymph node with central flow pattern (L) Spectral wave form pattern (C) and Vascular indices values (R) (Case-2)

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC)

Figure-11.Armamentarium

Figure-12.Collection of aspirates from right submandibular lymph node for FNAC in Supine position

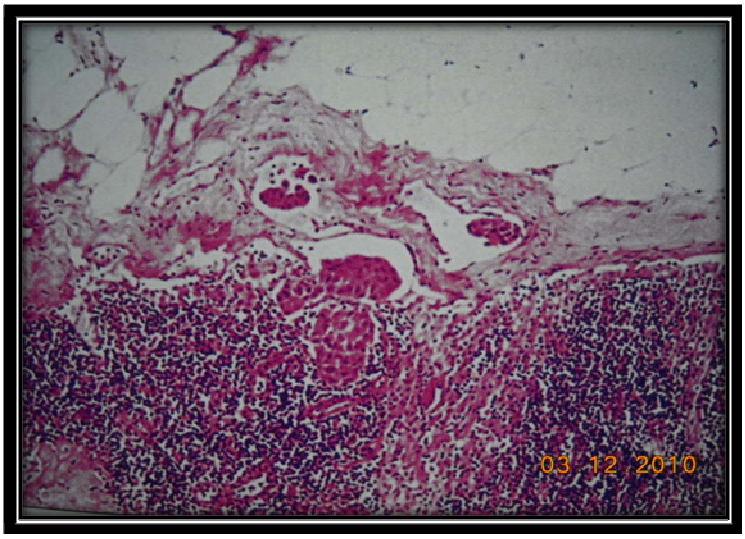


Figure-13.Photomicrograph showing metastatic lymph node with deposits of malignant squamous cells at multiple sites and rudimentary lymphoid tissue in sub-capsular area (H & E x10) (Case-1)

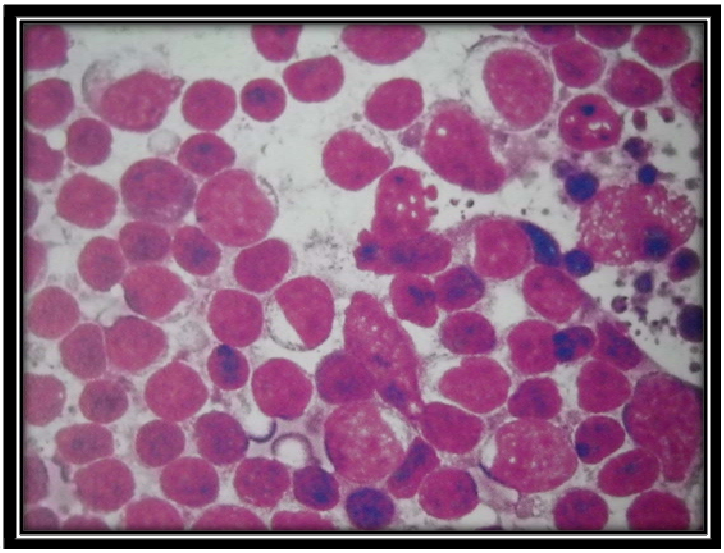


Figure-14.Photomicrograph showing reactive lymph node with centroblasts, centrocytes and small lymphocytes with nuclear fragments (MGG High Power) (Case-2)

TABLES AND CHARTS

Table–1. Gender wise distribution of study population(80 patients).

Gender	Group-I	Group-II	Total	P-value
Male	26(32.5%)	24(30%)	50(62.5%)	0.644
Female	14(17.5%)	16(20%)	30(37.5%)	
Total	40	40	80	

Table–2.Site-wise distribution of oral cancer

Site of cancer in oral cavity	Group-I	Group-II	Total	P-Value
Buccal mucosa	4(5%)	20(25%)	24(30%)	0.003**
Tongue	10(12.5%)	5(6.25%)	15(18.75%)	
Alveolus	15(18.75%)	7(8.7%)	22(27.5%)	
Oropharynx	3(3.75%)	1(1.25%)	4(5%)	
Gingivo buccal sulcus	-	3(3.75%)	3(3.75%)	
Retromolar trigone	2(2.5%)	2(2.5%)	4(5%)	
Palate	4(5%)	2(2.5%)	6(7.5%)	
Floor of mouth	2(2.5%)	0	2(2.5%)	
Total	40	40	80	

Table–3. Site-wise distribution of oral cancer with gender correlation.

Site of cancer in oral cavity	**Male**	**Female**	**Total**	**P-value**
Buccal mucosa	15(18.75%)	9(11.25%)	24(30%)	0.500
Tongue	11(13.75%)	4(5%)	15(18.75%)	
Alveolus	10(12.5%)	12(15%)	22(27.5%)	
Oropharynx	3(3.75%)	1(1.25%)	4(5%)	
Gingivo buccal sulcus	2(2.5%)	1(1.25%)	3(3.75%)	
Retromolar trigone	4(5%)	0	4(5%)	
Palate	4(5%)	2(2.5%)	6(7.5%)	
Floor of mouth	1(1.25%)	1(1.25%)	2(2.5%)	
Total	50(62.5%)	30(37.5%)	80	

Table–4.Group-wise distribution of TNM staging of oral cancer

TNM staging	Groups		Total	P- Value
	Group-I	Group-II		
T1	0	5(6.25%)	5 (6.25%)	<0.001**
T2	5(6.25%)	16(20%)	21(26.25%)	
T3	2(2.5%)	5(6.25%)	7(8.75%)	
T4	33(41.25%)	14(17.5%)	47(58.75%)	
Total	40	40	80	

Table–5. Site-wise distribution of oral cancer with TNM staging.

Site of cancer in oral cavity	TNM staging				Total	P-Value
	T1	T2	T3	T4		
Buccal mucosa	2(2.5%)	12(15%)	3(3.75%)	7(8.75%)	24	<0.001**
Tongue	0	1(1.25%)	0	14(17.5%)	15	
Alveolus	1(1.25%)	0	1(1.25%)	20(25%)	22	
Oropharynx	0	1(1.25%)	2(2.5%)	1(1.25%)	04	
Gingivo buccal sulcus	2(2.5%)	1(1.25%)	0	0	03	
Retro molar trigone	0	3(3.75%)	0	1(1.25%)	04	
Palate	0	3(3.75%)	1(1.25%)	2(2.5%)	06	
Floor of mouth	0	0	0	2(2.5%)	02	
Total	5 (6.25%)	21(26.25%)	7(8.75%)	47(58.75%)	80	

Table –6.Gender-wise distribution of TNM staging of oral cancer

Gender	TNM staging				Total	P-value
	T1	T2	T3	T4		
Male	3(3.75%)	16(20%)	4(5%)	27(33.75%)	50(62.5%)	0.514
Female	2(2.5%)	5 (6.25%)	3(3.75%)	20(25%)	30(37.5%)	
Total	5(6.25%)	21(26.25%)	7(8.75%)	47(58.75%)	80	

Table–7.Gender-wise distribution of cervical lymph nodes in 80 patients with oral cancer.

Gender	Group-I	Group-II	Total no. of lymph nodes	P-value
Male	44(39.3%)	26(23.2%)	70(62.5%)	0.398
Female	23(20.6%)	19(16.9%)	42(37.5%)	
Total	67(59.9%)	45(40.1%)	112	

Table-8.Comparison of clinical examination Vs FNAC of cervical lymph nodes in 80 oral cancer patients (112 lymph nodes)

Types of lymph nodes	Clinical suspicion	Fine needle Aspiration Findings (FNAC)	P-value
Metastatic nodes	67(59.9%)	84(75%)	0.010^{**}
Reactive nodes	45(40.1%)	28(25%)	
Total	112	112	

Table –9.Total cervical lymph nodes evaluated by CDUS in 80 patients with oral cancer

Groups	Clinically evaluated nodes	Additional nodes evaluated by CDUS	Total nodes evaluated by CDUS	P-Value
Group-I	67(51%)	17(13%)	84(64%)	0.013*
Group-II	45(34.5%)	2(1.5%)	47(36%)	
Total	112(85.5%)	19(14.5%)	131	

Table–10.Level-wise distribution cervical lymph nodes by CDUS

Levels of cervical lymph nodes	Group-I	Group-II	Total	P-value
I	67(51%)	45(34.4%)	112(85.4%)	0.086
II	12(9.1%)	2(1.6%)	14(10.7%)	
III	05(3.9%)	0	5(3.9%)	
Total	84(64%)	47(36%)	131	

Table –11.Correlation of Colour Doppler flow patterns of in 131 lymph nodes in 80 oral cancer patients

Colour Doppler Flow	**Group-I**	**Group-II**	**Total**	**P-value**
Central	6(4.6%)	29(22.1%)	35(26.7%)	<0.001**
Peripheral	64(48.8%)	17(13%)	81(61.8%)	
Mixed	11(8.3%)	1(0.9%)	12(9.2%)	
No flow	3(2.3%)	0	3(2.3%)	
Total	84(64%)	47(36%)	131	

Table-12. Mean values of Intranodal vascular resistance in metastatic and reactive lymph nodes

Vascular indices	**Metastatic nodes**		**Reactive nodes**		**P-value**
	Mean	**SD**	**Mean**	**SD**	
PI	2.33	0.78	0.92	0.32	<0.001**
RI	0.83	0.12	0.52	0.12	<0.001**

Table – 13.Correlation of vascular indices of CDUS in131 lymph nodes in 80 oral cancer patients

Vascular indices	**Group-I**	**Group-II**	**Total**	**P-value**
PI > 1.5 RI > 0.7	73(55.7%)	10(7.7%)	83(63.4%)	<0.001**
PI < 1.5 RI < 0.7	11(8.3%)	37 (28.3%)	48(36.6%)	
Total	84(64%)	47(36%)	131	

Table-14.Comparison of Colour Doppler flow pattern Vs FNAC in clinically evaluated cervical lymph nodes in oral cancer patients (112 lymph nodes)

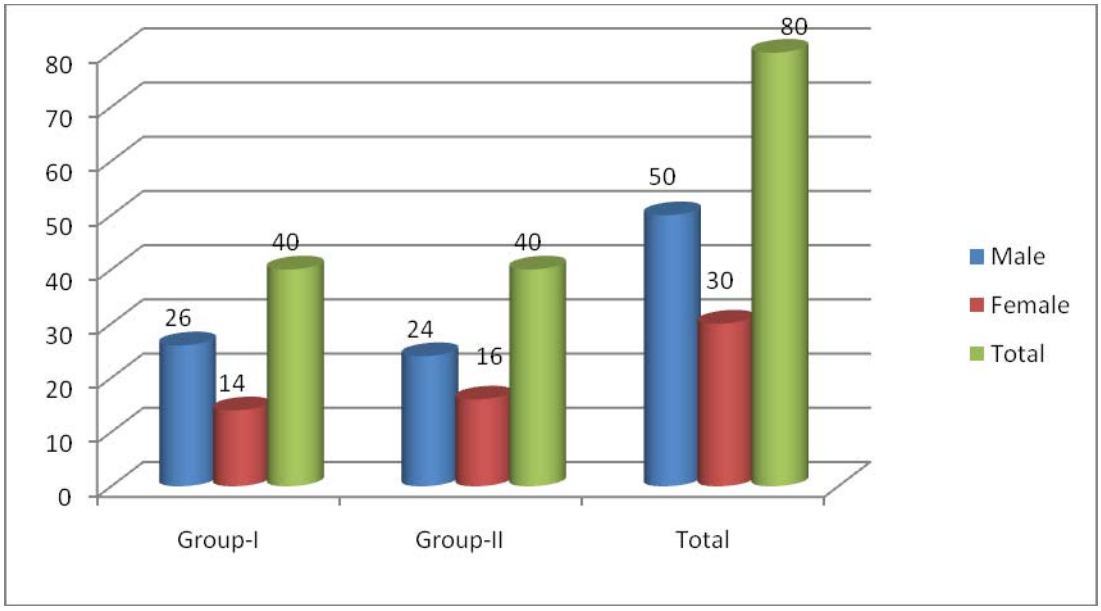
Types of lymph nodes	CDUS		Fine needle Aspiration Findings (FNAC)	P-value	
	Flow pattern (A)	Vascular indices (B)		(A)	(B)
Metastatic nodes	78(69.6%)	67(59.8%)	84(75%)	0.293	0.007**
Reactive nodes	34 (30.4%)	45(40.1%)	28(25%)		
Total	112	112	112		

Table-15.Comparison of Colour Doppler findings Vs Cytological examination of Cervical lymph nodes in 80 oral cancer patients (131 lymph nodes)

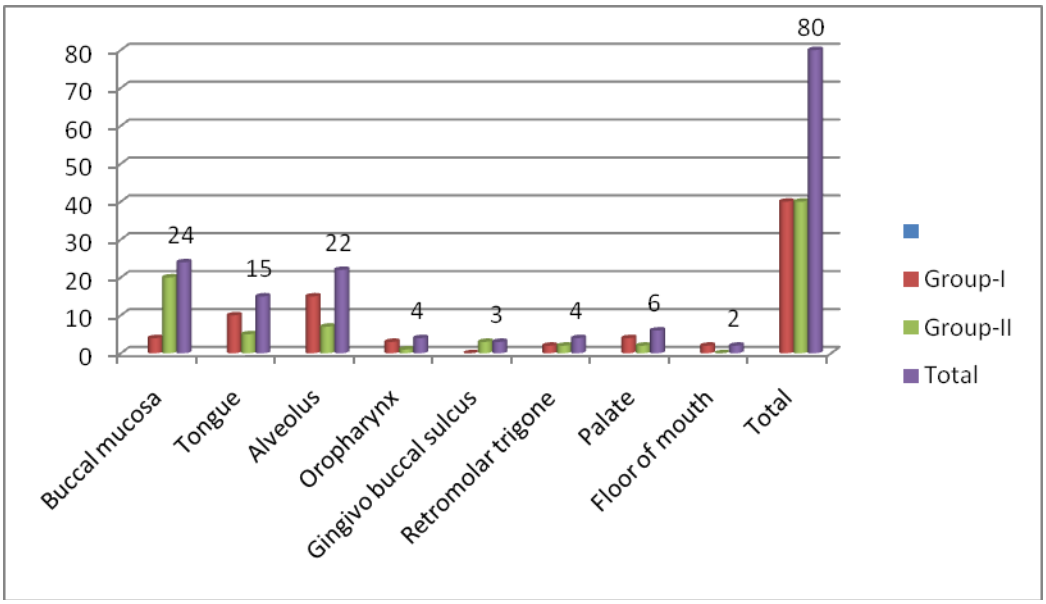
Types of lymph nodes	**CDUS**		**Fine needle aspiration Findings (FNAC)**	**P-value**	
	Flow pattern (A)	**Vascular Indices (B)**		**(A)**	**(B)**
Metastatic nodes	96(73.3%)	83(63.4%)	102(77.9%)	0.388	0.010**
Reactive nodes	35(26.7%)	48(36.6%)	29(22.1%)		
Total	131	131	131		

Charts

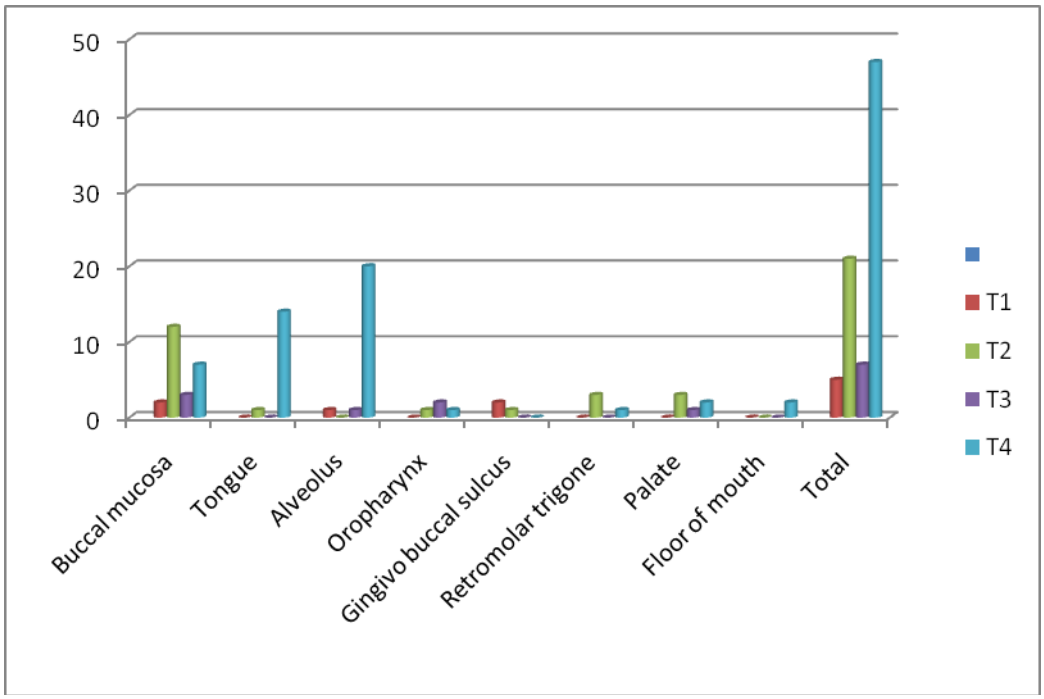
GENDER-WISE DISTRIBUTION OF STUDY POPULATION



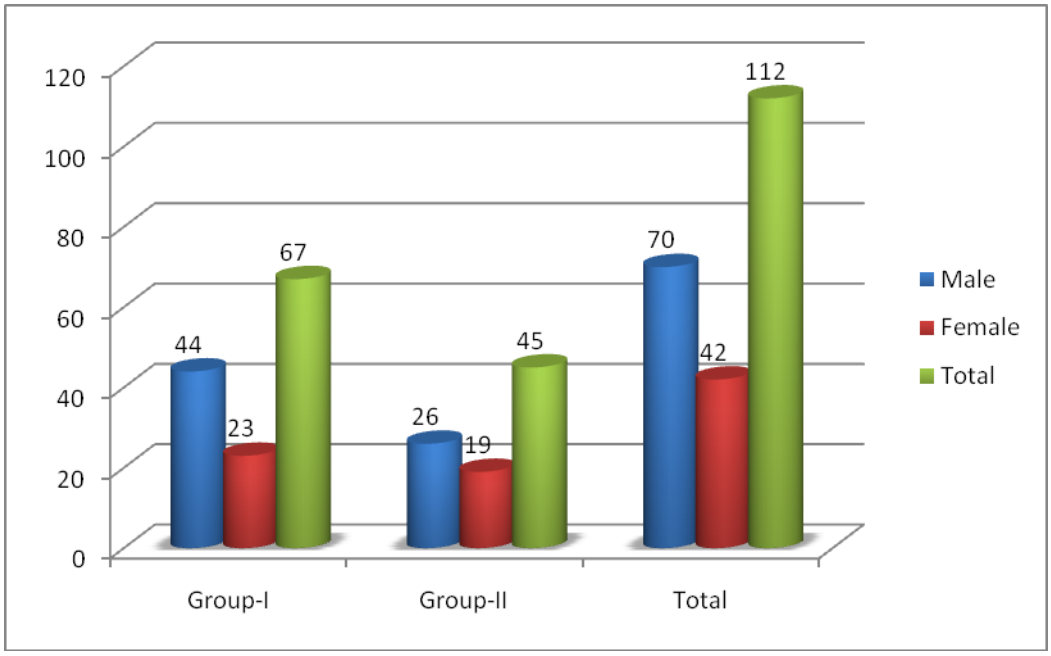
SITE-WISE DISTRIBUTION OF ORAL CANCER



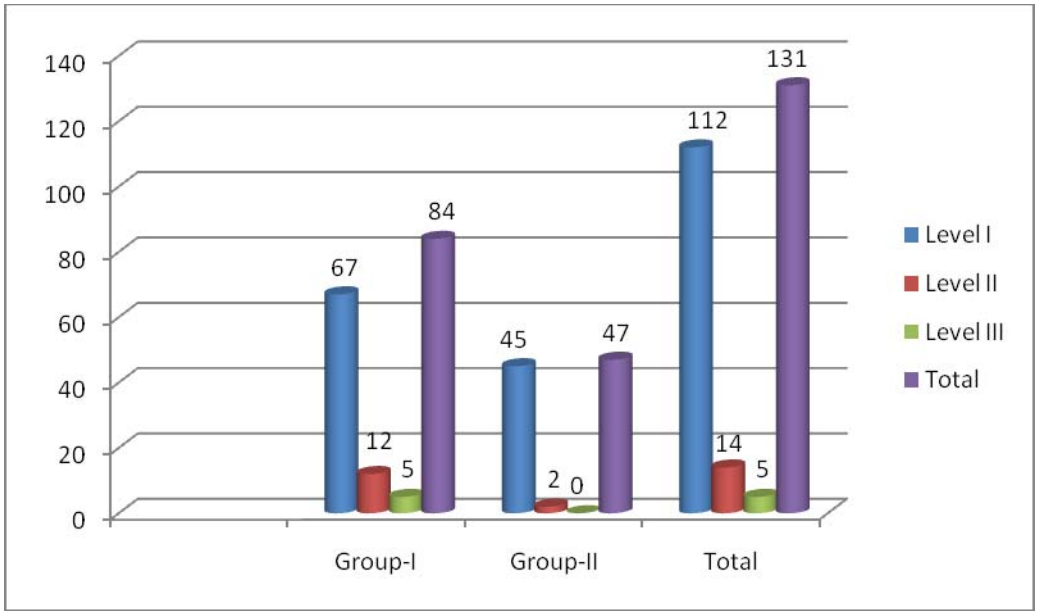
SITE-WISE DISTRIBUTION WITH TNM CORRELATION



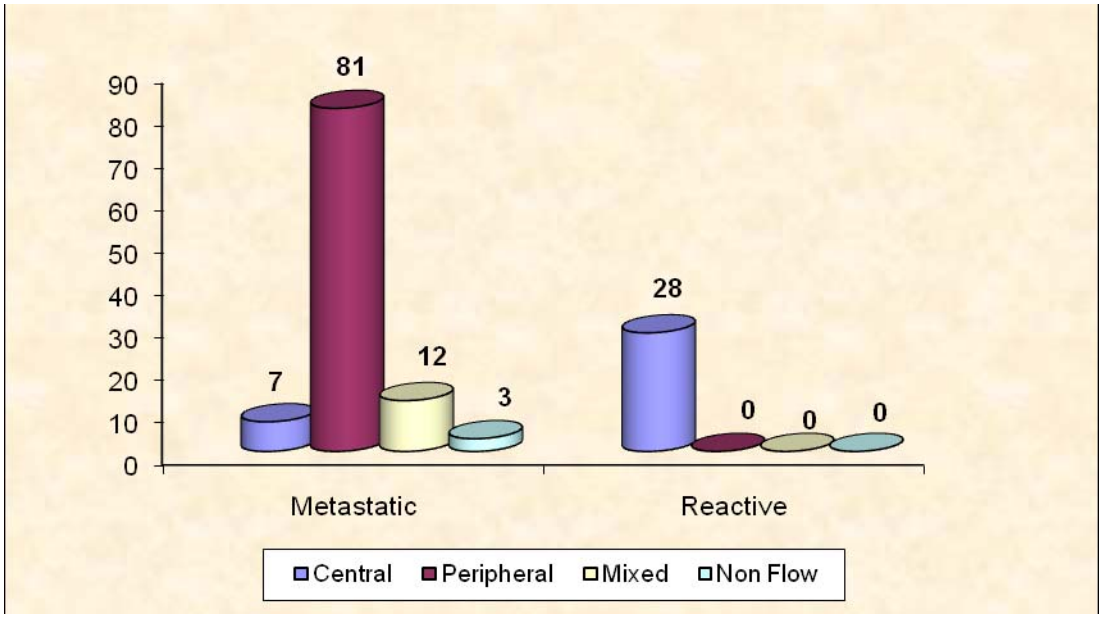
GENDER-WISE DISTRIBUTION OF LYMPH NODES



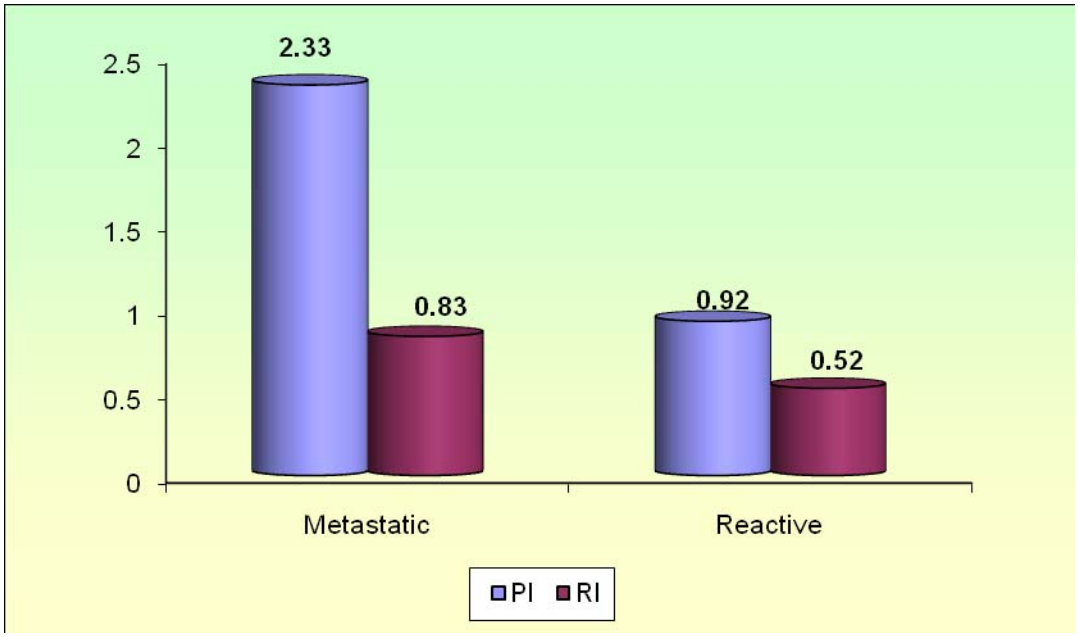
LEVEL-WISE DISTRIBUTION OF LYMPH NODES BY CDUS



CORRELATION OF COLOUR FLOW PATTERNS IN METASTATIC AND REACTIVE LYMPH NODES



MEAN VALUES OF INTRANODAL VASCULAR INDICES (PI & RI) IN METASTATIC AND REACTIVE LYMPH NODES



PI – PULSATILITY INDEX **RI -RESITIVITY INDEX**

RESULTS AND ANALYSIS

This study enrolled 80 subjects with oral cancer comprising two groups. Group-I included 40 patients with clinically suspected Metastatic Cervical lymph nodes, Group-II included 40 patients with clinically suspected Reactive Cervical lymph nodes based on the clinical criteria, with age range of 20-60 years either sex. The patients were selected from the Department of Oral Medicine and Radiology, Tamilnadu Government Dental College & Hospital, Chennai-600003. Group-I included 26 (65%) male (age range 32-60 years) and 14(35%) females (age range 25-60years). Group-II included 24(60%) male (age range 30-60 years) and 16(40%) females (age range 33-60years).So total male were 50(62.50%) and females were 30(37.50%) out of 80 patient. (Table-1)

Clinical evaluation of oral cancer

On clinical examination, out of 80 patients, the most common site of involvement by oral cancer were Buccal mucosa (30%), Alveolus (27.5%),Tongue (18.75%), Palate (7.5%), Oropharynx (5%), Retromolar trigone (5%), Gingivo-buccal-sulcus (3.7%), Floor of mouth (2.5%).

In group-I, out of 40 patients with clinically suspected metastatic lymph node, the most common site of oral cancer was

alveolus (15 patients). No patient had the involvement of gingivo-buccal sulcus. In Group-II, the most common site of oral cancer was buccal mucosa (20 patients). No patient had involvement of floor of mouth. (Table-2) There was a significant difference between the two groups regarding site of involvement of oral cancer (P-value=0.003**) (Table-2)

Gender-wise correlation of oral cancer with site of involvement

Out of 80 patients, 50 were males, 30 were females. In males the most common site of involvement was Buccal mucosa (18.75%), Tongue (13.75%), Alveolus (12.5%), Palate (5%), Retro-molar trigone (5%), Oropharynx (3.75%), Gingivo-buccal-sulcus (2.5%), and Floor of mouth (1.25%).

In females the most common site of involvement was Alveolus(15%), Buccal mucosa(11.25%),Tongue(5%), Palate (2.5%), Oropharynx (1.25%), Gingivobuccal-sulcus (1.25%), and Floor of mouth (1.25%).There was no significant difference between males and females regarding site involvement (P-value=0.500). (Table-3)

Correlation of TNM staging of Oral cancer within groups

Out of 80 cancer patient T4 stage was noted in 47(58.75%) patient, T3 in 7(8.75%) patient, T2 in 21(26.25%) patient and T1 in 5(6.25%) patient.

In Group-I, 33 (41.25%) patient had T4 stage, 5(6.25%) patients with T2 stage, 2(2.5%) patients with T3 stage. No patient was having T1 stage. In Group- II, T2 stage was noted in 16(20%) patients T4 stage was found in 14(17.5%) patients, T3 in 5(6.25%) patients, T1 in 5(6.25%) patients. On statistical analysis, there was a significant difference between groups regarding TNM staging (P-value $<0.001^{**}$) (Table-4)

Correlation of TNM staging with site of involvement of oral cancer

T4 stage was found most commonly involving alveolus in 20(25%) patient, tongue in 14(17.5%) patients , buccal mucosa in 7(8.75%) patients, palate and floor of mouth in 2(2.5%) patients each and oropharynx and retro molar trigone in 1(1.25%) patient each.

T2 stage was found in buccal mucosa in 12(15%) patients, palate & retro molar trigone in 3 (3.75%) patients each, tongue, oropharynx and gingivo-buccal sulcus in 1(1.25%) patient in each.

T3 stage was found most commonly at buccal mucosa in 3 (3.75%) patients, oropharynx 2(2.5%), alveolus and palate in 1(1.25%) patient each.

T1 involved buccal mucosa and gingivo-buccal sulcus in 2(2.5%) patients, alveolus in 1(1.2%) patient. There was a significant

relation between site of involvement and TNM staging (P-value<0.001**) (Table-5)

Although male had more T4 stage involvement but gender wise there was no significant difference between T staging in males and female (P-value=0.514)(Table-6)

Clinical evaluation of cervical lymph nodes in patient with oral cancer

Clinical examination detected total 112 palpable lymph nodes 80 patients with oral cancer. No. of nodes ranged from 1 to 3 in Group-I and from 1 to 2 in Group-II. Size of nodes ranged from 8x6mm in Group-II to 6x5cm in Group-I. Most commonly palpated nodes were Level I, followed by Level II and Level III. In Level I, the most common nodes involved were submandibular nodes. No node was palpated at Level IV or Level V. 13(16.2%) patient had bilateral lymph node involvement (9 in Group-I & 4 in Group-II).Most common site of oral cancer with bilateral lymph node involvement was tongue 5(38.5%) patients, alveolus 4 (30.8%) patients, buccal mucosa 3(23%) patients and palate 1(7.7%) patient.

On clinical evaluation out of those 112 nodes, 67(59.9%) were suspected to be metastatic in 40 patient of Group-I and 45(40.1%) were suspected to be reactive in 40 patient of Group-II based on

clinical criteria to differentiate metastatic lymph nodes from reactive nodes. No. of nodes involved in males and females in Group-I & Group-II was not significant (P-value=0.398) (Table-7)

Comparison of the results between Clinical and FNAC findings

Total 112 lymph nodes were evaluated clinically in both groups. Group-I included 67(59.9%) clinically suspected metastatic nodes in 40 patients with oral cancer. Group-II included 45(40.1%) clinically suspected reactive nodes in 40 patients with oral cancer.

A comparison of clinical findings with cytological findings in 112 clinically palpable cervical lymph nodes showed that out of 67 clinically suspected metastatic lymph nodes, 67 were confirmed for metastatic deposits (no false positive) and out of 45 clinically suspected reactive nodes 28(25%) lymph nodes proved reactive and 17(15.1%) proved metastatic by cytology (false negative).So total 84 (75%) nodes were diagnosed as metastatic and 28(25%) as reactive by FNAC. (Table-8).There was statistically significant difference between clinical diagnosis and FNAC findings (P-value=0.010**), accordingly a sensitivity of 79.8%, specificity of 100% with accuracy of 84.82% in patients with oral cancer.

Colour Doppler Ultrasonographic evaluation of cervical lymph nodes in patient with oral cancer

During CDUS examination of these 112 cervical lymph nodes in the same patients, an additional 19 (17 in Group-I and 2 in Group-II) lymph nodes were discovered which were also included in the overall CDUS study. So total lymph nodes evaluated by CDUS were 131(84(64%) in Group-I and 47(36%) in Group-II) .There was a significant difference between Group–I and Group-II regarding involvement of extra nodes. (P-value=0.013*) (Table-9)

Most commonly evaluated nodes were Level I, 112 (85.4%) nodes followed by Level II 14(10.7) and Level III, 5(3.9%) nodes. At Level, the most common node involved were submandibular in both groups (Total node=109, 65 in Group-I and 44 in Group-II). No node was evaluated at Level IV or Level V. On comparison between groups similar patterns of lymph node involvement were seen but in Group-II, there was no node involvement at Level III. There was no significant difference between two group regarding pattern of nodal involvement (P-value=0.086) (Table-10)

On the basis of colour flow pattern visible on colour Doppler ultrasound out of 131 lymph nodes 96(73.3%) [78 (59.4%)in Group-I and 18(13.9%) in Group-II] showed features of metastasis with

81(61.8%) nodes with peripheral flow [64(48.8%) in Group-I & 17 (13%) in Group-II], 12(9.2%) with mixed flow [11 (8.3%) in Group-I & 1(0.9%) in Group-II], 3(2.3%) with no flow (all in Group-I) and 35 (26.7%) showed features of reactive nodes with central vascular flow[6(4.6%) in Group-I & 29(22.1%)in Group-II].(Table-11). There was a significant difference between Group–I and Group-II regarding flow pattern. (P-value <0.001**)(Table-11)

On the basis of intranodal vascular resistance indices, In this study the Pulsatility indices from the vessels of metastatic lymph nodes ranged from 1.07 to 3.85, with a mean value of 2.33± 0.78(mean±SD).The Pulsatility indices from the vessels of reactive lymph nodes ranged from 0.43 to 2.76,with a mean value of 0.92±0.32(mean± SD).

The resistivity indices from the vessels of metastatic lymph nodes ranged from 0.44 to 0.94, with a mean value of 0.83± 0.12(mean±SD). The resistivity indices from the vessels of reactive lymph nodes ranged from 0.32 to 0.94, with a mean value of 0.52±0.12(mean± SD). There was statistically significant differences between the mean values of PI and RI for metastatic and reactive (p-value <0.001** for PI and RI) (Table-12).

On the basis of Pulsatility index (PI) & Resistivity index (RI)) provided by colour Doppler ultrasound out of 131 lymph nodes 83(63.4%) [73(55.7%) in Group-I and 10 (7.7%) in Group-II] showed features of metastasis (PI>1.5 & RI > 0.7) and 48 (36.6%) [11 (8.3%) in Group-I and 37 (28.3%) in Group-II] showed features of reactive nodes (PI <1.5 & RI <0.7). This study showed statistically significant differences between two groups regarding PI and RI values. (P-value <0.001**) (Table-13)

Fine needle aspiration cytology (FNAC) findings of cervical lymph nodes in patient with oral cancer

On Fine needle aspiration cytological confirmation of those 131 lymph nodes, the result was 102 (84 in Group-I & 18 in Group-II) were metastatic and 29(all in Group-II) were reactive.

Comparison of the results between clinical, CDUS and FNAC to find out the efficacy of CDUS

In the comparison of CDUS diagnosis with cytological diagnosis among 112 clinically evaluated cervical lymph nodes, it became evident that out of the 112 lymph nodes, 78(69.6%) [61 in Group-I & 17 in Group-II) were metastatic and 34(30.4%) [6 in Group-I & 28 in Group-II] were reactive by CDUS by colour flow pattern and 67(59.9%) [58 in Group-I & 9 in Group-II] were

metastatic and 45(40.1%) [9 in Group-I & 36 in Group-II] were reactive based on colour Doppler vascular indices as compared with clinical diagnoses of 67(59.9%) metastatic and 45(40.1%) reactive. FNAC diagnosed 84 (75%) nodes as metastatic and 28(25%) as reactive lymph nodes. (Table-14)

On comparison, there was no statistical difference between CDUS flow pattern and FNAC (P-value=0.293).but statistically significant difference was found between CDUS vascular indices findings and FNAC (P-value=0.007**) (Table-14)

CDUS study of those 131 lymph nodes (which include 112 clinically palpable lymph nodes and additional 19 lymph nodes discovered during CDUS), 96(73.3%) [78 in Group-I & 18 in Group-II)] had features of metastatic lymph nodes and the remaining 35(26.7%) lymph nodes (6 in Group-I & 29 in Group-II) had features of reactive lymph nodes. On the basis of vascular indices on CDUS of 131 nodes 83(63.4%) [(73 in Group-I & 10 in Group-II)] showed features of metastatic nodes and 48(36.6%) [(11 in Group-I & 37 in Group-II)] showed features of reactive nodes.

On Fine Needle Aspiration Cytological (FNAC) confirmation of those 131 lymph nodes, the result was 102(77.9%) [(84 in Group-I & 18 in Group-II)] were metastatic and 29 (22.1%) were reactive. On

comparison, there was no statistical difference between CDUS flow pattern and FNAC (P-value=0.388), but statistically significant difference was found between CDUS vascular indices findings and FNAC (P-value=0.010**) (Table-15)

DISCUSSION

This study was conducted in the Department of Oral Medicine and Radiology, Tamil Nadu Government dental college and hospital, Chennai-3; Barnard Institute of Radiology, Madras Medical College, Chennai-3; Goschen Institute of Pathology, Madras Medical College, Chennai-3.Study comprised 80 Adult Subjects with clinically and histopathologically diagnosed Oral cancer of either gender with age range of 20-60 years. To avoid over-diagnosis of lymph nodes in patients with oral cancer, study was divided in two groups based on clinical criteria. Group-I included 40 patients with clinically suspected Metastatic cervical lymph nodes, Group-II included 40 patients with clinically suspected Reactive Cervical lymph nodes.

All enlarged cervical lymph nodes in patients with oral cancer are traditionally assumed to be metastatic.[20,63,50] Recent studies have shown that there is a tendency to over-diagnose nodes as metastatic in patients with oral cancer and have suggested that all clinically palpable cervical lymph nodes in oral cancer patients cannot be assumed to be metastatic but there should be a re-evaluation of the clinical criteria for suspicion of cervical lymph nodes in patients with oral cancer.[28,29] So based on these studies, we divided study population into two groups, based on clinical criteria for

differentiating metastatic nodes from reactive nodes.[33,58] A total of 112 lymph nodes were evaluated in 80 patients with oral cancer, 67 nodes as clinically suspected metastatic in Group-I and 45 as clinically suspected reactive in Group-II.

CDUS correctly identified 112 cervical lymph nodes in 80 patients with oral cancer. During CDUS evaluation, an additional 19(17 in Group-I & 2 in Group-II) lymph nodes were discovered, which were either located in a clinically in-accessible region, deep seated, or in obese patients were obscured by the neck tissue.[19] This resulted in a false negative rate of 14.5% (19 lymph nodes) by clinical examination as those nodes were totally missed during clinical evaluation.

These findings supported the study by Bruneton et al[19] These 19 additional lymph nodes were found in 14 patients (12 in Group-I & 2 in Group-II).By FNAC confirmation, 18 out of 19 cervical lymph nodes had metastatic deposits so rate of occult metastasis were 94.7% which is different as compared to reported by Ahmed et al[2] and Suwarna et al as they found it to be 33%.[29] This study showed that in patients with oral cancer, highest risk for early metastasis to lymph nodes were to Level I (85.4%), followed by Level II (10.7%), Level III (3.9%). In level I, the most commonly nodes involved by oral

cancer were submandibular nodes (109 out of 112 level I nodes). These findings were comparable to the findings of Ying and Ahuja[78] and Suwarna[29] et al as the reported Level II and Level III nodes as most commonly involved. No nodes were palpated at Level IV or Level V. This findings was similar to the findings of Ying[78] and Ahuja and Suwarna et al.[29]

Most patients in this study with confirmed metastatic nodes on FNAC had T4 (47(58.75%) patients) stage of oral cancer at clinical presentation. And in T4 stage was the most common clinical presentation in patients with oral cancer involving the alveolus (out of 22 patient with alveolus involvement ,20 (90.9%) patients had T4 stage due to bone involvement as demonstrated by radiographs) followed by cancer of tongue(out of 15 patient with tongue involvement, 14(93.3%) patients had T4 stage).

So from these findings, this study supported the fact that the risk of metastasis to cervical lymph nodes increases with increasing T stage of oral cancer. Also endophytic tumours as well as tumours involving bone are more prone for metastasis than exophytic tumours. These findings were similar to the findings of Carew JF et al[20] and Suwarna et al.[29]

Colour Doppler flow Pattern

The role of Doppler ultrasound in the evaluation of metastatic nodes is based on the fact that tumours larger than a few millimeters in diameter stimulate the growth of new vessels. This tumour neo-vascularity has certain characteristics that enable a presumptive diagnosis of malignancy to be made. In evaluating nodal vascularity, Doppler ultrasound assesses two main features[53, 30, 5, 8]

1. Distribution of vessels within the node, and
2. Vascular parameters detecting intravascular resistance (RI, PI).

In this study, out of total 131 lymph nodes evaluated by CDUS, 81(61.8%) [64 (48.8%) in Group-I and 17(13%) in Group-II] showed peripheral color flow signal, all suggestive of metastatic nodes.

Out of these 81 nodes all were confirmed as metastatic nodes by FNAC. (False positive=zero).Our study again supported the fact that peripheral flow is suggestive of metastatic nodes which is similar to previous studies.[5, 16, 27].

In our study peripheral flow was not shown by FNAC proven reactive nodes. This finding was in contrast to previous studies[28,29]. This can be explained by difference in methodology as all patients were having oral cancer and patients were divided into two groups based on clinical criteria.

In metastatic lymph node involvement, destruction of hilar vascularity by tumour cells may result in the induction of vascular supply from the peripheral pre-existing vessels or from vessels in the peri-nodal soft tissue. Thus, they have peripheral flow.

Out of 131 nodes 35(26.7%) [6(4.6%) in Group-I and 29(22.1%) in Group-II] nodes showed central vascular flow, 33 nodes proved to be reactive and 2 metastatic, by FNAC.

Reactive nodes tend to have prominent hilar/central vascularity due to the increase in vessel diameter and blood flow as seen in 33 reactive nodes.[8] This finding was consistent with other studies, suggesting that presence of central flow is indicator of reactive nodes.[53,63,68,29,5,24] Presence of central flow in two metastatic lymph nodes was contradictory finding. This finding was comparable to the study by Sato et al[63] and Steinkamp et al[68].

The reason for this could be the presence of micro-metastases at the early stage of lymph node involvement, which could not be detected by CDUS as intranodal vascular alterations take place at a relatively late stage of metastasis.[68,71]

Another reason for central flow in metastatic nodes could be increased local immune reaction in the early stage of micro-infiltration, leading to increased vascularity. The original

architecture of the lymph node is rarely altered, leading to central vascular flow pattern in metastatic node.[74,78]

12(9.2%) [11(8.3%) in Group-I and 1(0.9%) in Group-II] lymph nodes showed mixed vascularity, which were confirmed as metastatic nodes by FNAC. This finding was consistent with the findings of Chih-Hsiu Wu et al[78] and SB Dangore et al[28].

Mixed vascular flow of the metastatic node can be explained by two pathogeneses. First, as the tumour nests replace the node, the pre-existing nodal vessels may be proliferated and transformed into feeding vessels by tumour angiogenesis, resulting in central aberrant nodal vessels. Second, advanced tumoural infiltration of a node will destroy the hilar blood supply, resulting in induction of the vascular supply from the peripheral pre-existing vessel or vessels in peri-nodal connective tissue, which may be accelerated by extra-capsular invasion.[53]This study showed no flow in 3(2.3%) FNAC proved metastatic cervical lymph nodes. This finding was consistent with the previous studies.[53,16,28]

There are studies that showed that absence of flow in a lymph node is an important CDUS feature suggesting its metastatic nature. Reason for no flow in metastatic nodes could be the total replacement

of the whole node by necrosed and keratinized tumour tissue leading to absence of vascular patterns.[63,68]

In our study no FNAC proven reactive nodes showed peripheral, mixed or no flow. This finding was in contrast to previous studies.[28, 29] This can be explained by difference in methodology as all patients were having oral cancer and patients were divided into two groups based on clinical criteria, which were not the case in previous studies.

On the basis of the presence of colour flow pattern, the sensitivity and specificity to differentiate between metastatic and reactive lymph node involvement in oral cancer patients were 94.1% and 100% respectively with a accuracy of 95.4%.As compared with a previous study, our study showed comparable sensitivity but high specificity and high accuracy.[28,29]

Overall results of colour Doppler flow criteria were highly statistically significant in this study (P<0.001).

Vascular parameters (PI & RI)

In the present study, higher values for both PI & RI were found in patients with metastatic lymph nodes, and lower values than the cut-off values for reactive nodes. These findings were consistent with most of the previous studies.[25,28,29,53,79]

Reason for higher values for metastatic nodes can be explained by the fact that as tumour cells spread into the lymph node, they grow and replace a large portion of the lymph node. Finally, the lymph node is totally replaced by tumour cells. At this stage, tumour cells compress vessels in the lymph node because of the limited space therein. This vascular compression by tumour cells increases vascular resistance.[8,25] The lower values found in reactive node are because of dilatation of blood vessels in reactive nodes.

The role of intravascular resistance parameters (RI, PI) in differentiating metastatic from reactive nodes has been a subject of controversy. Some studies have suggested that the vessels in a metastatic node had lower resistance (as reflected by a low RI) due to the absence of a muscle layer in tumour vessels and the presence of arterio-venous shunting.[5,22,68]

According to Ahuja et al[5] the reason for the contrasting results of the values of RI and PI in some studies is the differences in methodology and vessel sampling used for evaluating the RI and PI, such as (1) which node should be evaluated, and (2) how the RI and PI are calculated. In our study, the mean of randomly obtained RI and PI within the node was used.

Different cut-off points for RI and PI were used in previous studies[25,13,53,18,65] In our study, we applied the cut-off points suggested by Wu et al[73] (RI= 0.7 and PI=1.5) and found that the sensitivity and specificity in our population were 81.4% and 100% respectively with an accuracy of 85.5%, by applying those cut-off points. This also shows that flow pattern is more sensitive parameter when compared to vascular indices in differentiating metastatic nodes from reactive nodes. This finding is consistent with previous studies.[28,29]

The results of our study for these criteria showed that there was a statistically significant difference between the mean values of RI and PI for metastatic and reactive lymph nodes ($P<0.001$ for RI and $P<0.001$ for PI), which were comparable with previous studies by Steinkamp et al[68] and Shirakawa et al[65] while the findings of the other studies showed that differentiation between metastatic and lymph node enlargement is not possible solely on the basis of these Doppler indices.[53,9,22]

Comparison of Clinical and CDUS findings with FNAC as standard

As in our study, we used the clinical criteria to differentiate metastatic lymph nodes from reactive nodes in patients with oral cancer instead of using traditional assumption that each and every

enlarged lymph node is metastatic in patients with oral cancer, the false positive rate was zero i.e. there was no node which was clinically suspected as metastatic but shown reactive as by FNAC as a standard. So the specificity of clinical criteria 100%, with a sensitivity of 79.8% and accuracy of 84.8%.

Out of 112 clinically palpated nodes, CDUS showed 78 nodes as metastatic and 34 nodes as reactive by colour flow pattern and 67 as metastatic & 45 as reactive by vascular indices as compared to 84 metastatic nodes and 28 reactive nodes given by standard FNAC. False positive rate for metastatic nodes by CDUS flow pattern and by vascular indices were zero i.e. no reactive nodes were shown as metastatic by both. So the sensitivity of CDUS by vascular flow pattern was 92.85%, specificity of 100%, with accuracy of 94.6% and by vascular indices the sensitivity was 79.8%, specificity of 100%, and accuracy of 84.8%.

CDUS showed 96 nodes as metastatic and 35 nodes as reactive by colour flow pattern and 83 as metastatic & 48 as reactive by vascular indices as compared to 102 metastatic nodes and 29 reactive nodes given by standard FNAC. False positive rate for metastatic nodes by CDUS flow pattern and by vascular indices were zero i.e. no reactive nodes were shown as metastatic by both. So the

sensitivity of CDUS by vascular flow pattern was 94.1%, specificity of 100%, with accuracy of 95.4% and by vascular indices the sensitivity was 81.4%, specificity of 100%, and accuracy of 85.5%.

On comparison the CDUS flow pattern was more accurate in differentiating metastatic from reactive nodes in patients with oral cancer. Additionally 19 nodes were discovered by CDUS as compared to clinical palpation .This means that CDUS is more reliable than clinical examination and there is improvement in detection of node metastasis in oral cancer by CDUS as reported by previous studies.[53,80]

According to this study the overall rate of metastatic cervical nodes in oral cancer was 77.9% (102 out of 131 nodes were metastatic). This finding was comparable to study of Dietmar Koischwitz et al[43] they reported lymph node metastatic rate of 80% in patients with squamous cell carcinoma. However, Spiro JD, et al[67] and Dangore et al[29] reported a lower rate of lymph node metastasis (18.57%).

SUMMARY AND CONCLUSION

This study was conducted in the Department of Oral Medicine and Radiology, Tamil Nadu Government dental college and hospital, Chennai-3; Barnard Institute of Radiology, Madras Medical College, Chennai-3; Goschen Institute of Pathology, Madras Medical College, Chennai-3.Study comprised 80 Adult Subjects with clinically and histopathologically diagnosed Oral cancer of either gender with age range of 20-60 years. To avoid over-diagnosis of lymph nodes in patients with oral cancer, study was divided in two groups based on clinical criteria. Group-I included 40 patients with clinically suspected Metastatic cervical lymph nodes, Group-II included 40 patients with clinically suspected Reactive Cervical lymph nodes.

In summary our study support the fact that each node involved in patient with oral cancer should not be considered as metastatic, which can lead to unnecessarily treatment of neck.

Our study also showed that risk of metastasis is more in oral cancer patient with advance staging (T4 in 58.75% patients) as well as in patient with bone involvement.

In this study we found that most commonly involved cervical lymph nodes in patients with oral cancer are Level (85.4%) followed by Level II (10.7%) and Level III (3.9%). Bilateral lymph node was

more common with oral cancer involving tongue followed by alveolus.

Cervical lymph node involvement is a single most important factor in treatment and prognosis in patient with oral cancer, as regional metastasis decreases the survival rate significantly. So there is need to evaluate the neck thoroughly for any lymph node involvement in oral cancer patients which is not possible clinically (sensitivity=79.8%, specificity=100%, accuracy=84.8%) as our study have shown.

CDUS being a non-invasive, real-time procedure and easily applicable tool for the detection of alterations in the vasculature of lymph nodes may reduce the patient morbidity and therefore is of great clinical importance. In our study all of the findings suggested superiority of CDUS over clinical evaluation. In CDUS evaluation, flow pattern (sensitivity=94.1%, specificity=100%, accuracy=95.4%) is more accurate than the vascular indices (sensitivity=81.4%, specificity=100%, accuracy=85.5%) in differentiating metastatic from reactive lymph nodes in oral cancer patients.

From our study we conclude that CDUS plays a definitive role as an adjunct to clinical evaluation of differentiating metastatic from reactive cervical lymph involvement in patients with oral cancer as it

aid in grading and staging of oral cancer and can determine the treatment plan prognosis and morbidity by diminishing the possibility nodal dissection.

However, results of our study on diagnostic accuracy of Colour Doppler ultrasonography in evaluation of cervical lymph nodes in oral cancer patients can be validated with more studies involving large number of oral cancer patients.

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